Iprodione

DOCUMENT M-CA, Section 1

IDENTITY OF THE ACTIVE SUBSTANCE

Compiled by:

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CA 1 IDENTITY OF THE ACTIVE SUBSTANCE

Iprodione (BAS 610 F), a fungicide for foliar use in legumes and various other crops, is registered in Europe since many years. It has been fully reviewed under Directive 91/414/EEC and included in Annex I by Commission Directive No 2003/31/EC of 11 April 2003. Inclusion entered into force on 1 January 2004. Extension was granted until 31 October 2016 by Regulation No 823/2012/EU.

All relevant information on the first Annex I review including the endpoints used in environmental risk assessments can be found in the EU review report of iprodione 5036/VI/98-final (3 December 2002), the ECCO Full report on iprodione dated 01.10.2002 as well as in the iprodione Draft Monograph (June 1996) and the amendments thereto.

For the current registration renewal under Directive 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 / evaluations were initiated where considered necessary. According to Article 7(d) of Regulation (EU) No. 844/2012 this supplementary dossier includes data and risk assessments which were not part of the original dossier and which are necessary to reflect changes. Old data are presented as brief abstract.

Furthermore, a literature search was performed, and scientific publications were included into the dossier when considered relevant and being of sufficiently high quality. Hence, adequate summaries are provided in the appropriate dossier chapters.

CA 1.1 Applicant

BASF Agro B.V. Arnhem (NL) Zürich Branch
Im Tiergarten 7
8055 Zürich
Switzerland

Contact:

[redacted]
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Mobile No: [redacted]
E-mail address: [redacted]

Alternative:
[redacted]

Telephone No: [redacted]
Fax No:
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CA 1.2 Producer

Manufacturer of Iprodione (legal entity):

[redacted]

Contact:

[redacted]
Telephone No : [redacted]
Mobile No: [redacted]
E-mail address: [redacted]

Alternative:
[redacted]
Telephone No: [redacted]
Fax No:
E-mail address:

Location of manufacturing plant:

CONFIDENTIAL information – data provided separately (Document J)

CA 1.3 Common Name Proposed or ISO-accepted and synonyms

Iprodione

CA 1.4 Chemical Name (IUPAC and CA nomenclature)

IUPAC:	3-(3,5-dichlorophenyl)-N-isopropyl-2,4-dioxoimidazolidine-1-carboxamide
CA:	3-(3,5-dichlorophenyl)-N-(1methyl ethyl)-2,4-dioxo-1-imidazolidine carboxamide

CA 1.5 Producer's Development Code Numbers

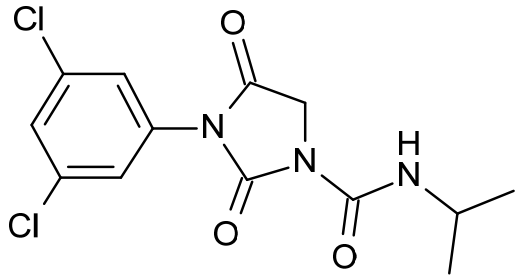
BASF Number:	BAS 610 F
BASF Registry Number:	Reg.No. 101169
BASF CL Number (old):	CL 182831

CA 1.6 CAS, EC and CIPAC Numbers

CAS Number:	36734-19-7
EINECS Number:	253-178-9
CIPAC Number:	278

CA 1.7 Molecular and Structural Formula, Molar Mass

Structural formula:



Molecular formula: $C_{13}H_{13}Cl_2N_3O_3$

Molar mass: 330.17 g/mol

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

Min. 960 g/kg, in accordance with 2003/31/EC (Annex I Inclusion Directive)

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

Iprodione TGAI does not contain any relevant impurities.

CA 1.11 Analytical Profile of Batches

CONFIDENTIAL information - data provided separately (Document J)

Iprodione

DOCUMENT M-CA, Section 2

PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE

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CA 2 PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.1 Melting point and boiling point			Melting Point: 134 °C Boiling Point: not relevant (solid at room temperature) (Information already reported and peer-reviewed previously; see Review Report, 5036/VI/98-final 3. December 2002)		Review Report Iprodione, 5036/VI/98-final 3. December 2002
CA 2.2 Vapour pressure, volatility	[see 2014/1085095 Kroehl T. 2014 a]	[see 2014/1085095 Kroehl T. 2014 a]	Vapour pressure: 5 ·10^{-7} Pa (for pure compound at 25 °C) 4 ·10^{-5} Pa (for pure compound at 51 °C) (Information already reported and peer-reviewed previously; see Review Report, 5036/VI/98-final 3. December 2002) The Henry's Law Constant is calculated with the data for vapour pressure and for water solubility. H = 1.8 x 10^{-8} kPa m^3 / mol. (***This report was mistakenly forgotten in BASF Application (October 2013), but is included since relevant for the requested information.)***	N	Review Report Iprodione, 5036/VI/98-final 3. December 2002 [see 2014/1085095 Kroehl T. 2014 a]

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.3 Appearance (Physical state, colour)			Appearance: White crystalline powder (Information already reported and peer-reviewed previously; see Review Report, 5036/VI/98-final 3. December 2002)		Review Report Iprodione, 5036/VI/98-final 3. December 2002
CA 2.4 Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity			UV/VIS: Maximum 204.5 nm, ε = 44333 L mol^{-1} cm^{-1}. At 295 nm in acetonitrile ε less than 10. (Information already reported and peer-reviewed previously; see Review Report, 5036/VI/98-final 3. December 2002) IR, NMR, MS: Reference spectra have been collected and are consistent with the chemical structure. (Information already reported and peer-reviewed previously; see Monograph, Volume 3 Annex B, June 1996)		Review Report Iprodione, 5036/VI/98-final 3. December 2002 Monograph, Volume 3 Annex B, June 1996

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.5 Solubility in water	[see 2013/1317196 Fieseler A.,Herrmann S. 2014 a]	[see 2013/1317196 Fieseler A.,Herrmann S. 2014 a]	Water solubility at 20°C: 9.0 mg/L (pure water) 8.9 mg/L (pH 5) 6.8 mg/L (pH 7) These values confirm the ones already peer-reviewed and reported in the Review Report : 13 mg/L at pH 3, 20°C 12.2 mg/L at pH 7, 20°C	Y	[see 2013/1317196 Fieseler A.,Herrmann S. 2014 a] Review Report Iprodione, 5036/VI/98-final 3. December 2002
CA 2.6 Solubility in organic solvents			Solubility at 20°C: hexane: 0.59 g/L toluene: 147 g/L n-octanol: 10 g/L acetonitrile: 168 g/L acetone: 342 g/L ethyl acetate: 225 g/L dichloromethane: 450 g/L (Information already reported and peer-reviewed previously; see Review Report, 5036/VI/98-final 3. December 2002)		Review Report Iprodione, 5036/VI/98-final 3. December 2002

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.7 Partition co-efficient n-octanol/water			Log P_{OW}: 2.99 (at pH 3), 3.0 (at pH 5) (Information already reported and peer-reviewed previously; see Review Report, 5036/VI/98-final 3. December 2002)		Review Report Iprodione, 5036/VI/98-final 3. December 2002
CA 2.8 Dissociation in water • dissociation constant(s) (pKa values) • identity of dissociated species • dissociation constant(s) (pKa values) of the active principle			Not relevant (no protonation; no dissociation occurs in water). (Information already reported and peer-reviewed previously; see Review Report, 5036/VI/98-final 3. December 2002)		Review Report Iprodione, 5036/VI/98-final 3. December 2002
CA 2.9 Flammability and self-heating	EC A.10 EC A.16	Batch COD-001260 97.8%	Not highly flammable. No self-heating was detected.	Y	[see 2012/1227385 Achhammer 2013 a]

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.10 Flash point			Not applicable, as the test substance has a melting point well above 40 °C.		
CA 2.11 Explosive properties	OECD Guideline 113 (DSC)	Batch COD-001260 97.8%	Not explosive. (Information already reported and peer-reviewed previously; see Review Report, 5036/VI/98-final 3. December 2002) Results of additional Differential Scanning Calorimetry analysis: 1st reaction: Onset temperature: 185 °C Peak temperature: 207 °C Energy release: 10 J/g 2nd reaction: Onset temperature: 285 °C Peak temperature: 346 °C Energy release: 800 J/g 3rd reaction: Onset temperature: 415 °C Peak temperature: 454 °C Energy release: 90 J/g	Y	Review Report Iprodione, 5036/VI/98-final 3. December 2002 [see 2012/1227385 Achhammer 2013 a]

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.12 Surface Tension			Surface tension: 73.0 nN/m (20 °C, 6 mg/L iprodione) (Information already reported and peer-reviewed previously; see Monograph, Volume 3 Annex B, June 1996)		Monograph, Volume 3 Annex B, June 1996
CA 2.13 Oxidising properties	EC A.17	Batch COD-001260 97.8%	Not an oxidizing substance.	Y	[see 2012/1227385 Achhammer 2013 a]
CA 2.14 Other studies					

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Active substance

DOCUMENT M-CA, Section 3

FURTHER INFORMATION ON THE ACTIVE SUBSTANCE

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CA 3 FURTHER INFORMATION ON THE ACTIVE SUBSTANCE

CA 3.1 Use of the Active Substance

Iprodione is a contact fungicide with mainly preventive activity belonging to the group of dicarboximide fungicides. One key benefits of iprodione is its broad range of disease control. Iprodione provides excellent control of diseases caused by the following pathogens: *Botrytis, Sclerotinia, Alternaria, Monilinia, Mycosphaerella, Phoma, Fusarium, Helminthosporium, Pyrenophora, Rhizoctonia, Corticium, Pleiochaeta* and *Septoria* spp.

CA 3.2 Function

Iprodione is used as a fungicide to control a broad spectrum of diseases in all relevant specialty crops, including grapes, fruits and nearly all commercially relevant vegetables.

CA 3.3 Effects on Harmful Organisms

Iprodione is a contact fungicide belonging to the group of the dicarboximides. The mode of action of the dicarboximides has been widely investigated and a variety of possible modes of action have been suggested. The most common theory was that dicarboximides induce membrane lipid peroxidation triggered by active oxygen species within susceptible fungal cells but recent studies suggest a link with an osmoregulatory pathway.

The majority of Iprodione is not taken up into the plant, but acts on the leave surface as preventive compound. The principal effect is achieved by controlling the disease at the beginning of its development.

CA 3.4 Field of Use Envisaged

Agriculture

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

The complete list of currently registered uses can be found in document D-2.

Iprodione is used in a wide range of crops including:
Vegetables

- Brassica Vegetables
  - Head cabbage
  - Leafy cabbage
  - Flowering cabbage
  - Brussel sprouts
- Bulb Vegetables
  - Onions (incl. spring onions)
  - Shallot
  - Garlic
- Root & Tuber Vegetables
  - Carrots
  - Potato
  - Radish & Parsnip
- Stem Vegetables
  - Leek
  - Celery
  - Asparagus
  - Fennel
  - Rhubarb
- Fruiting Vegetables
  - Cucurbits with edible peel (Cucumber, Gherkins, Courgette)
  - Cucurbits with inedible peel (Melon, Pumpkin, Zucchini)
  - Tomato, Aubergine, Pepper
- Leafy Vegetables & Herbs
  - Lettuce & similar
  - fresh herbs
  - Cichory, witloof

Legume crops

- Dry pulses (dry harvest)
  - beans: field beans,
  - peas: chickpeas, field peas, chickling vetch
- Legume vegetables (fresh harvest)
  - beans (with & without pods)
  - peas (with & without pods)

Biannual / perennial crops
- Berries & Small Fruits
  - Strawberries
  - Currants
  - Raspberries
- Pome & Stonefruit
- Citrus fruits
- Grapes

Others
- Ornamentals
- Tree nursery

Iprodione is used to control a broad range of most important fungal diseases such as:
- *Botrytis spp.*
- *Alternaria spp.*
- *Sclerotinia spp.*
- *Rhizoctonia spp.*
- *Stemphylium spp.*
- *Monilinia spp.*
- *Penicilium spp.*

CA 3.6 Mode of Action

Iprodione belongs to the dicarboximide group of fungicides. The mode of action of the dicarboximides has been widely investigated and a variety of possible modes of action have been suggested. These were reviewed by Edlich and Lyr (1995) and Yamaguchi and Fujimara (2005). The most common theory was that dicarboximides induce membrane lipid peroxidation triggered by active oxygen species within susceptible fungal cells but recent studies suggest a link with an osmoregulatory pathway. Dicarboximides interfere with the osmotic signal transduction pathway consisting of histidine kinase and MAP kinase cascades (Cui *et al*. 2002, Leroux *et al*. 2002, Oshima *et al.* 2002, Cui *et al*. 2004). Cui *et al.* suggested that dicarboximides bind to the "coiled-coil region" of a putative osmosensing histidine kinase orthologous to *os-1* in *Neurospora crassa* (Cui *et al*. 2002, Cui *et al*. 2004).

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

After intensive use, a certain degree of resistance can be determined for most active ingredients. In the case of Iprodione, resistant isolates were found with *Botrytis cinerea* in grapevines and crops such as strawberries, protected crops or ornamentals. In other fungal species, for example *Fusarium nivale* and *Sclerotinia homoecarpea* in turf*, Monilinia fructicola* and *Alternaria* spp. resistance to dicorboximides has been found only sporadically without causing practical problems. Further information can be found on the homepage of the "Fungicide Resistance Action Committee (FRAC)".

On the basis of resistance risk, the following use recommendations and resistance management strategies had been defined:

In the case of *Rhizoctonia solani* and Iprodione, the combined risk is low. No specific resistance management strategies are therefore required.

However, in the case of *Botrytis cinerea* and iprodione the resistance risk assessment has indicated a high risk. In the case of *Monilinia* spp.*, Alternaria* spp.*, Stemphylium vesicarium*, *Sclerotinia* spp. and Iprodione, a medium risk is indicated. In these cases above, resistance management strategies are needed.

An important resistance management strategy is the restriction of use. These restrictions are recommended and summarised by FRAC.

BASF is a member of the FRAC Dicarboximide Expert Forum and has participated in forming the guidelines for management strategy. The general recommendations for resistance management of dicarboximides are shown on the web site cited in the references and can be summarised as follows:

- Minimise the selection pressure by minimising the number of applications. As a guide, do not apply more than two to three per crop per season.
- Restrict applications to those times when *Botrytis* infection pressure is high.
- Maintain regular prolonged times without exposure to dicarboximides.
- Where resistance is well established, use combinations to stabilise *Botrytis* control, but their application must follow the same rules as for dicarboximides alone.

For more information, go to the FRAC website (www.frac.info).

Depending on crop and pathogen BASF recommends a maximum of 1 - 4 application per season. Furthermore BASF recommends to use Iprodione only as an integral part of spray programmes against all target pathogens.

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

Report: CA 3.8/1
Anonymous, 2013a
Safety data sheet - Iprodione technial 96%
2013/1402552
Guidelines: EEC 1907/2006
GLP: no

The safety data sheet contains detailed information and advice on methods and precautions concerning handling, storage, transport or fire, based on scientific tests.

CA 3.9 Procedures for Destruction or Decontamination

Report: CA 3.9/1
Anonymous, 2013a
Safety data sheet - Iprodione technial 96%
2013/1402552
Guidelines: EEC 1907/2006
GLP: no

The safety data sheet contains detailed information and advice on procedures for destruction or decontamination, based on scientific tests.

CA 3.10 Emergency Measures in Case of an Accident

Report: CA 3.10/1
Anonymous, 2013a
Safety data sheet - Iprodione technial 96%
2013/1402552
Guidelines: EEC 1907/2006
GLP: no

The safety data sheet contains detailed information and advice on emergency measures in case of an accident, based on scientific tests.

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Iprodione

DOCUMENTM-CA, Section4

ANALYTICAL METHODS

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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

Report:	CA 4.1.1/1 Daum A., 2006a Determination of the active ingredient Iprodione in technical grade active ingredient of BAS 610 F, and the formulations BAS 610 06 F, BAS 610 10 F and BAS 610 11 F by HPLC 2007/1006102
Guidelines:	none
GLP:	no

Report:	CA 4.1.1/2 Daum A., 2007a Validation of the analytical method AFL0732/01 2007/1006103
Guidelines:	EPA 830.1800, EEC 96/46, SANCO/3030/99 rev. 4 (11 July 2000), CIPAC 3807 (improved version), OECD Principles of Good Laboratory Practice, GLP Principles of the German Chemikaliengesetz (Chemicals Act)
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Principle of the method
Method AFL0732/01 describes the determination of the active ingredient content in iprodione TGAI and formulations. The method is based on CIPAC method 278 but without using internal standard.
After preparation the samples are analysed by RP-HPLC with UV-detection. Quantitation is achieved by external standard calibration using authentic reference items with known content.

Applicability of CIPAC methods
CIPAC method 278 is available for the analysis iprodione active ingredient in TC, WP, SC and WG formulations by HPLC, using the internal standard method.

Identity
The identity of iprodione active ingredient (Reg.-No. 101169) was proved by comparison of the retention time of the reference item in the calibration solutions with the retention time of the test items. During the spectroscopic investigations for the specificity the identity was also confirmed by MS data.

Specifity (selectivity)
Specificity of method AFL0732/01 was ensured by comparison of retention times of the corresponding peaks of the test items and reference item. No co-eluation was observed.

Linearity
The evaluation of the linearity data yielded linear dependence of the HPLC-UV responses from the analyte iprodione. The coefficient of correlation was found to be greater than 0.999.

Concentration range [mg/L]:	189.6 – 595.9
Slope [ng/Response]:	0.0004357
y-Axis intercept [ng]:	-82.32
Correlation coefficient:	0.999923

Precision (repeatability)
The precision (repeatability) of method AFL0732/01 was performed by six independent replicate sample determinations. The results can be seen in the table below. 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

Test Item	Nominal Conc. [%]	corresp. concentr. 'C'	%RSDR (Inter Lab. RSD)	%RSDr (Intra Lab. RSD)	%RSD analyzed	%RSD Accepted
Iprodione TGAI	100.00	1.0000	2.00	1.34	0.45	yes

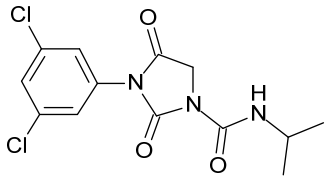
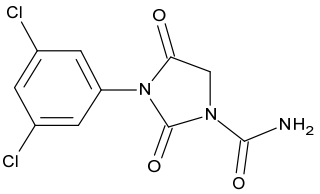
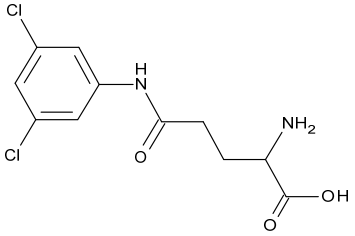
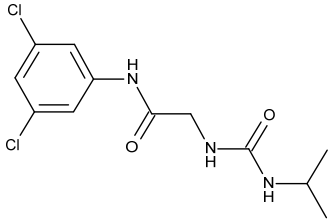
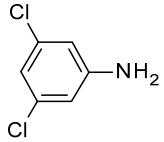
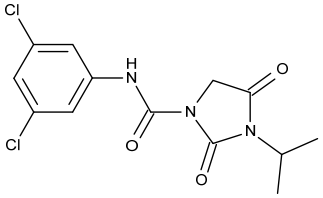
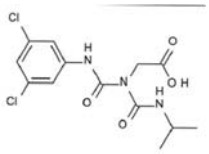
Conclusion
The results of the present validation study showed that method AFL0732/01 is suitable for the determination of the active ingredient content in iprodione TGAI.

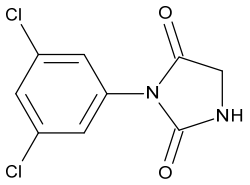
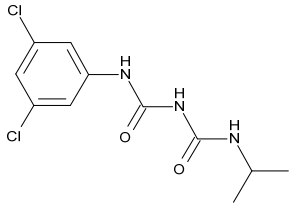
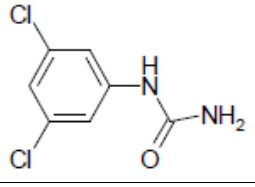
(b) Determination of significant and relevant impurities and additives (such as stabilisers) in the active substance as manufactured

CONFIDENTIAL information - data provided separately (Document J)

CA 4.1.2 Methods for risk assessment

A concordance list of structures and designations of reference compounds mentioned in CA 4.1.2 and 4.2 as well as the respective sections of Doc N is given below.

Compound designation [1)]	Reference code (Reg. No.)	Formula	Molecular weight (g/mol)	Structure
Iprodione RP 26019 M610F000	101169	$C_{13}H_{13}Cl_2N_3O_3$	330.17	
RP 32490	5079628	$C_{10}H_7Cl_2N_3O_3$	288.09	
M610F007	5916256 (L-Form)	$C_{11}H_{12}Cl_2N_2O_3$	291.13	
RP 37176	5079612	$C_{12}H_{15}Cl_2N_3O_2$	304.18	
RP 32596, 3,5-DCA M610F012	85831	$C_6H_5Cl_2N$	162.02	
RP 30228 M610F001	5079647	$C_{13}H_{13}Cl_2N_3O_3$	330.17	
RP 35606	5079626	$C_{13}H_{15}Cl_2N_3O_4$	348.2	

Compound designation [1)]	Reference code (Reg. No.)	Formula	Molecular weight (g/mol)	Structure
RP 25040 M610F004	207099	$C_9H_6Cl_2N_2O_2$	245.1	
RP 36221 M610F002	5079618	$C_{11}H_{13}Cl_2N_3O_2$	290.15	
LS 720942	89517	$C_7H_6Cl_2N_2O$	205.0	

1) For RP codes, different formats are in use:
"RP xxxxx" or or "RPxxxxx" or "RP0xxxxx"
independent from these format differences, the last 5 digit "xxxxx" are unique for every compound

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

The following methods cover the compounds that have to be considered for the discussion on environmental risk assessment as summarized in Document N, chapter 8.5 and discussed in CA 7.4:

Soil: Iprodione, RP 35606, RP 30228, RP 25040, RP 36221 and dichloroaniline
Sediment: Iprodione, RP 30228, RP 35606
Water: Iprodione, RP 35606, RP 30228, RP 36221, dichloroaniline , RP 25040 and LS 720942.
Air: Iprodione

Soil

Report:	CA 4.1.2/1 Walter W.,Horton W., 2013a Determination of Iprodione metabolites Reg.Nos. 207099, 5079618, 89517 (formerly RP 25040, RP 36221, LS720942) and 85831 (Dichloroaniline) in soil 2013/1003162
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Principle of the methods

The BASF Method allows the determination of iprodione metabolites with Reg. Nos.: 207099, 5079618, 89517 and dichloroaniline (Reg. No. 85831) in soil. Residues were shaker extracted from soil specimens once using acidified (0.1% formic acid) acetonitrile/water (1:1, v/v) and two more times with acetonitrile containing 0.1% formic acid. The extracts were placed in an ultrasonic bath, centrifuged and filtrated. An aliquot of the combined extracts was diluted (10x) and directly analysed by mean of liquid chromatography with tandem mass spectrometric detection (LC-MS/MS). The limit of quantitation (LOQ) of the method is 0.005 mg/kg for each analyte.

Recovery findings

The analytical method was developed for the determination of iprodione metabolites with Reg. Nos.: 207099, 5079618, 89517 and dichloroaniline (Reg. No. 85831) in soil.
All single mean recovery values (5 replicates) were in the acceptable range of 70 - 110% for both mass transitions validated. Detailed results of recoveries for each mass transition and soil are given in Table 4.1.2-1.

Table 4.1.2-1: Results of method validation: 207099, 5079618, 89517 and dichloroaniline in soil specimens

Soil	Analyte	m/z	No. of replicates	Fortification level [mg/kg]	Mean Recovery [%]	RSD [%]
LUFA 2.2	Reg No. 207099	245 → 162	5	0.005	105	4
			5	0.05	110	2
		243 → 160	5	0.005	94	7
			5	0.05	110	2
	Reg No. 5079618	290 → 205	5	0.005	95	8
			5	0.05	103	3
		290 → 127	5	0.005	104	8
			5	0.05	106	3
	Reg No. 89517	207 → 127	5	0.005	97	6
			5	0.05	104	5
		205 → 127	5	0.005	97	2
			5	0.05	104	2
	Reg No. 85831 (Dichloroaniline)	164 → 129	5	0.005	87	12
			5	0.05	90	6
		162 → 74	5	0.005	84	12
			5	0.05	92	6
Fiorentino Poggio Renatico 1	Reg No. 207099	245 → 162	5	0.005	95	7
			5	0.05	110	1
		243 → 160	5	0.005	101	5
			5	0.05	109	1
	Reg No. 5079618	290 → 205	5	0.005	97	6
			5	0.05	110	3
		290 → 127	5	0.005	92	9
			5	0.05	110	4
	Reg No. 89517	207 → 127	5	0.005	91	6
			5	0.05	110	2
		205 → 127	5	0.005	102	7
			5	0.05	103	2
	Reg No. 85831 (Dichloroaniline)	164 → 129	5	0.005	82	19
			5	0.05	110	5
		162 → 74	5	0.005	85	13
			5	0.05	88	12

Linearity	Good linearity (regression coefficients ≥ 0.99) was observed in the range of 0.010 ng/mL to 10 ng/mL for the four iprodione metabolites.
Specificity	The method allows the specific determination of iprodione metabolites with Reg. Nos.: 207099, 5079618, 89517 and dichloroaniline (Reg. No. 85831) in soil in general. This was demonstrated on the basis of two different soils. Each compound could be determined at two different mass transitions. Apparent residues in all blank control samples were below 20% of the LOQ. No interferences from soil components or from reagents, solvents and glassware were observed.
Limit of Quantitation	The limit of quantitation defined by the lowest fortification level successfully tested was 0.005 mg/kg for all analytes.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 4.1.2-1:.
Reproducibility	Reproducibility of the method was not determined within this validation study.
Conclusion	**The method for analysis of iprodione metabolites with Reg. Nos.: 207099, 5079618, 89517 and dichloroaniline (Reg. No. 85831) uses HPLC-MS/MS for final determinations, which is a modern and highly specific technique. The limit of quantitation was 0.005 mg/kg for each analyte.** **It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantitation and recoveries and is therefore applicable to correctly determine residues of the four iprodione metabolites in soil.**

The following study (2013/1400376) is submitted instead of 2013/1311298 as mentioned in the application previously (also mentioned with the wrong title naming water instead of soil). For completeness reasons it was decided to include all 3 analytes in one validation study as presented here.

Report:	CA 4.1.2/2 Heinz N.,Class T., 2013a Development and validation of an analytical method for the determination of Iprodione degradate/intermediate Reg.No. 5079626 (formerly RP35606), of Iprodione isomer Reg.No. 5079647 (formerly RP30228) and of Iprodione (BAS 610 F) in soil 2013/1400376
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 Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Principle of the methods

A 10-g soil aliquot (W) was extracted with 50 mL (V_{Ex}) of acetonitrile/water (7/3, v/v, 50 µL formic acid added) by mechanical shaking for 15 min. Then the soil extract was centrifuged (5 min, 4000 rpm) and an aliquot of 6.0 mL (V_1) was measured into another vial.
20 mL of water were added and the mixture was extracted 3-times, each time with 5 mL of dichloromethane. The combined organic extract was reduced to dryness under a stream of nitrogen at about 40 °C. Then the residue was re-dissolved in 3 mL (V_{End}) of acetonitrile or acetonitrile/water (1/1 v/v, 0.1 % formic acid) for determination of the analytes by LC-MS/MS.
For all analytes (Iprodione Degradate/Intermediate Reg. No. 5079626, of the Iprodione Isomere Reg. No. 5079647 and of Iprodione (BAS 610 F, Reg. No. 101169) two parent-daughter ion transitions were used for quantitation and confirmation.

Recovery findings

The method was proved to be suitable for determining Iprodione Degradate/Intermediate Reg. No. 5079626, of the Iprodione Isomer Reg. No. 5079647 and of Iprodione (BAS 610 F, Reg. No. 101169) in soil using LC-MS/MS with a limit of quantitation (LOQ) of 0.005 mg/kg and a limit of detection (LOD) of 0.0015 mg/kg or less than 30% of the LOQ. Validation experiments were conducted in two different soils. The mean recovery values for the three analytes were between 70% and 110% with the exception of the Iprodione isomer in the soil LUFA 5M at higher fortification level with 114%. For the iprodione intermediate, the 10-fold LOQ level fortifications were for both soils and for both MS/MS ion transitions in the range of 60 and 69%, however, excellent relative standard deviations of ≤ 11% do these recoveries make acceptable. The detailed results are given in Table 4.1.2-2.

Table 4.1.2-2: Validation results of a residue analytical method for Iprodione Degradate/Intermediate Reg. No. 5079626 for the Iprodione Isomer Reg. No. 5079647 and for Iprodione (BAS 610 F, Reg. No. 101169) in two soils

Soil	Analyte	m/z	No. of replicates	Fortification level [mg/kg]	Mean Recovery [%]	RSD [%]
LUFA 5M	Iprodione	330 → 245	5	0.005	100	11
		332 → 247	5	0.005	106	10
		330 → 245	5	0.05	105	8
		332 → 247	5	0.05	108	6
	Reg. No. 5079626 (Iprodione Degradate/Intermediate)	348→ 263	5	0.005	91	10
		348 → 76	5	0.005	86	15
		348→ 263	5	0.05	68	11
		348 → 76	5	0.05	65	11
	Reg.No.5079647 (stable isomer)	330 → 101	5	0.005	103	15
		332 → 101	5	0.005	99	11
		330 → 101	5	0.05	109	4
		332 → 101	5	0.05	114	7
LUFA 2.2	Iprodione	330 → 245	5	0.005	108	6
		332 → 247	5	0.005	106	7
		330 → 245	5	0.05	107	4
		332 → 247	5	0.05	106	5
	Reg. No. 5079626 (Iprodione Degradate/Intermediate)	348→ 263	5	0.005	100	6
		348 → 76	5	0.005	99	10
		348→ 263	5	0.05	69	7
		348 → 76	5	0.05	60	9
	Reg.No.5079647 (stable isomer)	330 → 101	5	0.005	102	10
		332 → 101	5	0.005	101	5
		330 → 101	5	0.05	104	5
		332 → 101	5	0.05	99	6

Linearity

Good linearity (regression coefficients ≥ 0.99) was observed in the range of 0.50 ng/mL to 50 ng/mL for the three analytes.

Specificity

The method allows the specific determination Iprodione Degradate/Intermediate Reg. No. 5079626, of the Iprodione Isomer Reg. No. 5079647 and of Iprodione (BAS 610 F, Reg. No. 101169), in soil. This was demonstrated on the basis of two different soils. Each compound could be determined at two different mass transitions. Apparent residues in all blank control samples were below 30% of the LOQ.

Limit of Quantitation The limit of quantitation (LOQ) was 0.005 mg/kg for each analyte.

Repeatability The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 4.1.2-2.

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

Conclusion

The method for analysis of the Iprodione degradate/intermediate Reg. No. 5079626, of the Iprodione isomer Reg. No. 5079647 and of Iprodione (BAS 610 F, Reg. No. 101169) uses LC-MS/MS for final determination, which is a modern and highly specific technique. The limit of quantitation was 0.005 mg/kg for each analyte

It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantitation and recoveries and is therefore applicable to correctly determine residues of the Iprodione degradate/intermediate Reg. No. 5079626, of the Iprodione isomer Reg. No. 5079647 and of Iprodione (BAS 610 F, Reg. No. 101169) in soil.

Water

Report:	CA 4.1.2/3 Jooss S., 2013a Determination of Iprodione metabolites Reg.Nos. 207099, 5079618, 89517 (formerly RP 25040, RP 36221, LS 720942) and 85831 (Dichloroaniline) in ground and surface water 2013/1003163
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Principle of the methods

The BASF Method allows the determination of iprodione metabolites with Reg. Nos.: 207099, 5079618, 89517 and dichloroaniline (Reg. No. 85831) in surface and ground water. Water specimens were acidified with formic acid and analysed by mean of liquid chromatography with tandem mass spectrometric detection (DI-LC-MS/MS). The limit of quantitation (LOQ) of the method is 0.03 µg/L for each analyte.

Recovery findings

The analytical method proved to be suitable to determine the iprodione metabolites with Reg. Nos.: 207099, 5079618, 89517 and dichloroaniline (Reg. No. 85831) in surface and ground water with an LOQ of 0.03 µg/L. Validation experimetns were conducted in surface and ground water. All average recovery values (mean of 5 replicates per fortification level and analyte) were between 70% and 110%. The detailed results are given in Table 4.1.2-3.

Table 4.1.2-3: Results of method validation: 207099, 5079618, 89517 and dichloroaniline in water specimens

Analyte	m/z	Matrix	Replicates	Fortification level [µg/L]	Mean recovery [%]	RSD [%]
Reg. No. 207099	**245→162**	roundwater	5	0.03	82	6
				0.3	74	4
		Surface water	5	0.03	90	5
				0.3	81	6
	243→160	Groundwater	5	0.03	79	10
				0.3	75	6
		Surface water	5	0.03	82	9
				0.3	82	5
Reg. No. 5079618	**290→205**	Groundwater	5	0.03	103	7
				0.3	107	4
		Surface water	5	0.03	105	6
				0.3	94	7
	290→127	Groundwater	5	0.03	100	6
				0.3	102	6
		Surface water	5	0.03	97	9
				0.3	105	3
Reg. No. 89517	**207→127**	Groundwater	5	0.03	89	6
				0.3	81	3
		Surface water	5	0.03	93	10
				0.3	90	3
	205→127	Groundwater	5	0.03	87	5
				0.3	83	7
		Surface water	5	0.03	87	5
				0.3	91	4
Reg. No. 85831	**164→129**	Groundwater	5	0.03	85	16
				0.3	80	16
		Surface water	5	0.03	79	11
				0.3	81	11
	162→74	Groundwater	5	0.03	109	14
				0.3	107	12
		Surface water	5	0.03	76	13
				0.3	85	11

Linearity	Good linearity ($r \geq 0.99$) was observed in the range of 0.009 µg/L to 1.0 µg/L for the two ion transitions of the four metabolites in the two different water types (groundwater and surface water), respectively.
Specificity	The method successfully determines the iprodione metabolites with Reg. Nos.: 207099, 5079618, 89517 and dichloroaniline (Reg. No. 85831) in water in general. This was demonstrated on the basis of two different water types (groundwater and surface water) using the highly specific detection technique HPLC/MS-MS with two ion transitions for each analyte. No interferences (> 30% of the limit of quantitation) in the tested untreated samples from water components or from reagents, solvents and glassware were observed at the retention times and ion transitions of each analyte.
Limit of Quantitation	The limit of quantitation defined by the lowest fortification level successfully tested was 0.03 µg/L for all analytes.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 4.1.2-3.
Reproducibility	Reproducibility of the method was not determined within this validation study.
Conclusion	**The method for the analysis of iprodione metabolites with Reg. Nos.: 207099, 5079618, 89517 and dichloroaniline (Reg. No. 85831) in groundwater and surface water uses HPLC-MS/MS for final determination, which is a modern and highly specific technique. The limit of quantitation is 0.03 µg/L.** **It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantitation and recoveries and is therefore applicable to correctly determine the iprodione metabolites in groundwater and surface water samples.**

Report:	CA 4.1.2/4 Class T., 2013a Determination of Iprodione (BAS 610 F, Reg.No. 101169), Iprodione degradate/intermediate Reg.No. 5079626 (formerly RP35606) and Iprodione isomer RP30228 (Reg.No. 5079647) in ground and surface water 2013/1311332
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 Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Principle of the methods

Initially a direct injection (DI)-LC-MS/MS was assessed for all three analytes, which separated all three analytes chromatographically and detected each of them with two different MS/MS mass transitions. For this method 1.0 mL of the water sample (adjusted to pH 5 with formic acid) was directly injected into the liquid chromatograph and analysed by LC-MS/MS for Iprodione and its intermediate RP35606. For the iprodione isomer RP30228 (Reg. No. 5079647) in water samples the sensitivity of the (DI-)LC-MS/MS method was about 10-times worse than its parent isomer.
Thus, for this analyte (and additionally for iprodione parent) a second method was developed, using C^{18}–based solid-phase extraction (SPE) of 100-mL water samples, followed by elution of the analytes with 2.5 mL acetonitrile and subsequent LC-MS/MS analysis of the enriched water extracts.

Recovery findings

The methods proved to be suitable to determine all three analytes in water, with an LOQ of 0.03 µg/L. Validation experiments were conducted in surface and ground water. All average recovery values were between 70% and 110%. The detailed results are given in Table 4.1.2-4 and Table 4.1.2-5.

Table 4.1.2-4: Results of the method validation for the determination of Iprodione and the Iprodione degradate / intermediate Reg.No. 5079626 (formerly RP35606) in ground water and surface water with the DI-LC-MS/MS method

Analyte	m/z	Matrix	Replicates	Fortification level [µg/L]	Mean recovery [%]	RSD [%]
Iprodione Reg.No. 101169	330→245	ground water	5	0.03	98	3
				0.3	102	3
		surface water	5	0.03	98	5
				0.3	100	4
	332→247	ground water	5	0.03	99	4
				0.3	100	4
		surface water	5	0.03	104	2
				0.3	101	3
Reg.No. 5079626	348→76	ground water	6	0.03	99	6
				0.3	94	6
		surface water	5	0.03	95	3
				0.3	92	3
	348→263	ground water	6	0.03	94	8
				0.3	93	8
		surface water	5	0.03	96	2
				0.3	91	4

Table 4.1.2-5: Results of the method validation for the determination of Iprodione and the Iprodione isomer RP30228 (Reg.No. 5079647) in ground water and surface water with the SPE-LC-MS/MS method

Analyte	m/z	Matrix	Replicates	Fortification level [µg/L]	Mean recovery [%]	RSD [%]
Iprodione Reg.No. 101169	330→245	ground water	5	0.03	99	5
				0.3	101	5
		surface water	5	0.03	100	2
				0.3	103	4
	332→247	ground water	5	0.03	100	4
				0.3	101	5
		surface water	5	0.03	99	4
				0.3	103	5
RP30228 Reg.No. 5079647	330→101	ground water	5	0.03	101	3
				0.3	104	4
		surface water	5	0.03	97	3
				0.3	102	4
	332→101	ground water	5	0.03	99	3
				0.3	104	4
		surface water	5	0.03	97	2
				0.3	100	6

Linearity

Good linearity ($r \geq 0.99$) was observed for the two mass transitions in the two different water types (ground water and surface water).

Specificity

Both methods were successfully validated and provide a limit of quantitation (LOQ) of 0.03 µg/L per analyte. This was demonstrated on the basis of two different water types (ground water and surface water) using DI-LC-MS/MS and C^{18}-SPE LC-MS/MS and monitoring two ion transitions for each analyte. No interferences (> 30% of the limit of quantitation) in the tested untreated samples from water components or from reagents, solvents and glassware were observed at the retention times and ion transitions of each analyte.

Limit of Quantitation

The method had a limit of quantitation (LOQ) of 0.03 µg/L for each analyte.

Repeatability

The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 4.1.2-4 and Table 4.1.2-5.

Reproducibility

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

Conclusion

Two different method procedure were developed for the determination of the Iprodione degradate/intermediate with the BASF Reg. No. 5079626 (formerly RP35606) and of Iprodione (BAS 610 F, Reg. No. 101169) and its isomer RP30228 (Reg. No. 5079647) in ground and surface water. Both methods uses HPLC-MS/MS for final determination, which is a modern and highly specific technique. The limit of quantitation is 0.03 µg/kg for all analytes, respectively.

It could be demonstrated that the methods fulfil the requirements with regard to specificity, repeatability, limit of quantitation and recoveries and are therefore applicable to correctly determine Iprodione, the intermediate with the Reg.No. 5079626 and the isomer RP30228 in ground water and surface water samples.

Air

The following study is included in this dossier for completeness reasons and was not mentioned in the application previously.

Report:	CA 4.1.2/5 Dorn U., 2000a Validation of an analytical method for the determination of Iprodione in air R014656
Guidelines:	EEC 96/46, EEC 91/414, SANCO/825/00 rev. 6 (20 June 2000)
GLP:	yes (certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Principle of the method

The method AR 251-00 was developed for the determination of iprodione in air samples. The analyte was spiked to Tenax adsorption tubes. After sucking air through the glass tubes for 6 hours at 35°C and a relative humidity of 94%, the tube content was extracted three times with ethyl acetate. Final determination of iprodione was achieved by GC/ECD. As confirmatory method GC with EI full scan MS can be used.

Recovery findings

During the method validation the extraction efficiency, the stability of the adsorbed iprodione on storage for 5 days at room temperature and in the freezer and the recovery and safe sampling volume without breakthrough with warm, humid air (35°C, 94 % rel. humidity) were examined. The results from the fortification experiments at two spiking levels (1.0µg /2.0 µg/m^3 and 100 µg / 200 µg/m^3) showed that the recoveries were between 70% and 110%.

In the course of the sampling experiments with warm humid air, at each fortification level, the average recovery was in the range 70 – 110 % (see Table 4.1.2-6).

Table 4.1.2-6: Results of the method validation for the determination of Iprodione in the air

Test System	Analyte	Replicates		Fortification level [µg]	Mean recovery [%]	RSD [%]
Air	iprodione	Extraction efficiency	5	1.0	97	
			5	100	103	
		Storage Stability (5 days, RT)	5	1.0	88	
			5	100	87	
		Storage Stability (5 days, -23°C)	5	1.0		
			5	100		
		Recovery after Sampling of warm, humid air	5	1.0	87	6
			5	100	90	4

Linearity Good linearity (R^2 > 0.99) was observed in the range of 0.005 ng/µL to 0.10 ng/µL for iprodione.

Specificity Under the described conditions method AR 251-00 is specific for the determination of iprodione in air. Significant interferences (> 30% of LOQ) were not observed at the retention time and mass-to-charge ratio of iprodione ions.

As confirmatory method GC with EI full scan MS can be used.

Limit of Quantitation The method achieves a limit of quantitation (LOQ) of 2.0 µg/m^3 iprodione in air.

Repeatability The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 4.1.2-6.

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

Conclusion **Method AR 251-00 was validated at concentrations of 2.0 µg/m^3 air and 200 µg/m^3 air. This method fulfils the requirements with regard to specificity, repeatability, limit of quantitation and recoveries and is therefore applicable to correctly determine residues of iprodione in air.**

Additionally a validated method for Parent + RP 30228 in water and soil is provided as supplemental information (2007/1039647):

Report:	CA 4.1.2/6 Schulz H.,Meyer M., 2007a Determination of Iprodione and its metabolite RP30228 in water and soil - Validation of the method 2007/1039647
Guidelines:	SANCO/825/00 rev. 7 (17 March 2004), OECD-DOC ENV/MC/CHEM(98)17 Paris 1998
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

The aim of the study was to perform a validation of an analytical method for the determination of iprodione and its metabolite RP30228 in water (tap water and surface water) and in soil (LUFA type 2.1).

Principle of the methods

Water:
Both analytes were extracted from water using dichloromethane. After concentration to dryness and reconstitution, the final determination was performed by HPLC-MS/MS. Two fragment ions were used for evaluation. The limit of quantitation (LOQ) of the method was 0.05 µg/L in water for both analytes.

Soil:
Both analytes were extracted from soil using acetonitrile/water (70/30). An aliquot of the extract was extracted with dichloromethane. After concentration to dryness and reconstitution, the final determination was performed by HPLC-MS/MS. Two fragment ions were used for evaluation. The limit of quantitation (LOQ) of the method was 0.010 mg/kg in soil for both analytes.

Recovery findings

The method proved to be suitable to determine iprodione and its metabolites RP30228 in water, with an LOQ of 0.05 µg/L and in soil with an LOQ of 0.010 mg/kg. Validation experiments were conducted in tap and surface water as well as in one soil. All average recovery values (mean of 5 replicates per fortification level and analyte) were between 70% and 110%. The detailed results are given in Table 4.1.2-7

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Table 4.1.2-7: Results of the method validation for the determination of iprodione and its metabolite RB30228 in surface water, ground water and soil

Analyte	m/z	Matrix	Replicates	Fortification level [µg/L]	Mean recovery [%]	RSD [%]
Iprodione	330.0→244.9*	Tap water	5	0.05 0.5	93.0 94.2	7.8 2.4
		Surface water	5	0.05 0.5	99.9 91.7	7.5 5.5
		Soil	5	0.010 0.10	95.5 105.1	5.9 5.9
	332.0→246.9	Tap water	5	0.05 0.5	97.0 89.3	6.9 3.7
		Surface water	5	0.05 0.5	106.7 96.9	3.6 4.7
		Soil	5	0.010 0.10	97.1 106.3	4.1 5.6
RB30228	330.0→100.8*	Tap water	5	0.05 0.5	95.4 99.7	6.2 2.6
		Surface water	5	0.05 0.5	91.8 84.1	5.9 3.1
		Soil	5	0.010 0.10	86.7 106.3	4.4 8.2
	332.0→100.8	Tap water	5	0.05 0.5	101.1 92.8	4.6 2.4
		Surface water	5	0.05 0.5	93.7 87.4	4.6 8.9
		Soil	5	0.010 0.10	92.7 111.1	3.6 4.5

* used for quantitation

Linearity

Good linearity ($r \geq 0.99$) was observed in the range of 0.02 ng to 0.7 ng for the ion transition which was used for the quantitation of iprodione and its metabolite RB30228. Only calibration curves for drinking water (tap water) was shown in the report.

Specificity

The method successfully determines iprodione and its metabolite RB30228 in water and soil. This was demonstrated on the basis of two different water types (tap water and surface water) using HPLC-MS/MS, and monitoring two ion transitions for each analyte. No interferences (> 30% of the limit of quantitation) in the tested untreated samples from water components or from reagents, solvents and glassware were observed at the retention times and ion transitions of each analyte.

Limit of Quantitation	The method had a limit of quantitation of 0.05 µg/L in water for both analytes and of 0.010 mg/kg in soil for both analytes.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were below 20% in all matrices. The detailed values are shown in Table 4.1.2-7.
Reproducibility	Reproducibility of the method was not determined within this validation study.
Conclusion	**The method for the analysis of iprodione and its metabolite RB30228 in tap water, surface water and soil uses HPLC-MS/MS for final determination, which is a modern and highly specific technique. The limit of quantitation is 0.05 µg/L in water and 0.010 mg/kg in soil.** **It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantitation and recoveries and is therefore applicable to correctly determine iprodione and its metabolite RB30228 in tap water, surface water and soil samples.**

(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 Iprodione 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 exposure studies were conducted with Iprodione, 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

The following methods cover the compounds necessary for the discussion on the residue definition for risk assessment for products of plant and animal origin as summarized in Document N, chapter 7.3 and discussed in CA 6.7:

Plant:	Iprodione x conversion factor 1.2 (accounting 10% each for RP30228 and RP32490)
Animal:	
in milk and kidney:	Sum of iprodione and all metabolites containing the 3,5-dichloroaniline or 3,5-dichloro-4-hydroxyaniline moieties, expressed as iprodione
in other animal matrices:	Sum of iprodione and all metabolites containing the 3,5-dichloroaniline moiety, expressed as iprodione

Plant

The methods for which validation data are provided below were used for data generation in the submitted residue trials.
A summary of the method can be found in the respective section of Doc N, chapter 5.1.3.
The first method has been peer reviewed previously during the Annex I inclusion process and is added here for completeness only.

Bourgade C. et al (1997)

Full study reference: Bourgade C. et al (1997): Iprodione: Analytical method for the determination of residues in plant products
BASF DocID R014615 (1997/1003298)

Principle of the method

Residues are extracted from the plant samples by macerating with acetone. The extracts are cleaned up by liquid-liquid partition with dichloromethane.
For the purification step, the substrates are divided into 3 groups:
Group 1 (fruits, vegetables and cereals): the extracts are purified on a Florisil cartridge.
Group 2 (difficult fruits and vegetables: i.e. grapes, carrots, leeks): the extracts are purified on a Diol cartridge.
Group 3 (oil products: oilseeds and tree nuts): the extracts are washed with n-hexane then purified on a Diol cartridge.
The quantitation is carried out by gas chromatography on a semi capillary column, using an electron capture detector and external standardization.

Recovery findings

The average recovery in all matrices was between 70 and 110% except in lettuce at a spiking level of 10.0 mg/kg (64%).

Table 4.1.2-8: Validation data for analytical method AR 144-97 for the determination of BAS 610 F residues in food of plant origin

Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No of analyses
Lettuce (group 1)	0.02 2.0 10.0	87 82 64	2 3 4	3 3 3
Grapes (group 2)	0.02 2.0 10.0	75 88 76	1 2 3	3 3 3
Sunflower seeds (group 3)	0.02 0.10 0.30	84 75 78	1 2 1	3 3 3

Linearity The detector response was found to be linear in the range of 20 to 200 µg/L.

Specificity No interferences due to reagents were detected. Interferences due to substrates were <30% LOQ.
A selected ion monitoring GC-MS technique could be used as a confirmatory method [see R014658 (2004/1021136) Fuchsbichler G. 2000] and [see R014659 Laporte F. 2000 a].

Limit of Quantitation The method achieves a limit of quantitation (LOQ) of 0.02 mg/kg iprodione.

Repeatability The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 4.1.2-8.

Conclusion **For each substrate and at each level, the mean recovery complies with the EU criteria (between 70% and 110%, except for lettuce at 10.0 mg/kg). The repeatability, expressed as the relative standard deviation (RSD) was lower than 20% and the LOQ was 0.02 mg/kg for iprodione.**

Additionally a preliminary version of this method (C025678) was used in some of the earlier trials which were previously evaluated on EU level and the information was therefore not included in this dossier.

While the original method (R014615) has been evaluated previously, the supporting confirmatory method using GC-MS for 1997/1003298 is included here:

Report:	CA 4.1.2/7 Fuchsbichler G., 2000a Demonstration of GC/MS as confirmatory chromatographic method for the detection of Iprodione in plant products R014658
Guidelines:	EEC 96/46, SANCO/825/00 rev. 6 (20 June 2000)
GLP:	yes (certified by Bayerisches Staatsministerium fuer Arbeit, Familie und Sozialordnung, Muenchen)

Report:	CA 4.1.2/8 Laporte F., 2000a Demonstration of GC/MS as confirmatory chromatographic method for the detection of Iprodione plant products R014659
Guidelines:	EEC 96/46, SANCO/825/00 rev. 6 (20 June 2000)
GLP:	yes (certified by Bayerisches Staatsministerium fuer Arbeit, Familie und Sozialordnung, Muenchen)

This study represents the confirmatory method to method AR 144-97 summarized above.

Principle of the method

The purpose of the study was to demonstrate that GC-MS proposed as confirmatory chromatographic technique in the analytical method AR 144-97 [see R014615 (1997/1003298) Bourgade C. et al. 1997] could be effectively used as such.
The original method was applied without major modifications on 3 groups of substrate: fruit, vegetables and cereals (group 1), difficult fruit and vegetables (group 2) and oily products such as oil seeds and tree nuts (group 3).
Selective ion monitoring was used for detection and quantitation.

Recovery findings

The average recovery in all matrices was between 70 and 110%.

Table 4.1.2-9: Confirmatory validation data for analytical method AR 144-97 for the determination of BAS 610 F residues in food of plant origin

Matrix	Fortification level [mg/kg]	Recovery [%]	No of analyses
Lettuce	0.02	85	1
Grapes	0.02	96	1
Wheat (grain)	0.02	94	1
Sunflower (seed)	0.02	97	1

Linearity A linear calibration curve was established in the range 0.10 µg/mL to 0.50 µg/mL with a correlation coefficient $R^2 > 0.99$.

Specificity The level of interference measured in the reagent blanks and control samples was <30% LOQ.
Iprodione was identified using two fragment ions with a m/z ratio >100. A third fragment ion was not used due to matrix interference.

Limit of Quantitation The method achieves a limit of quantitation (LOQ) of 0.02 mg/kg iprodione.

Repeatability The relative standard deviations (RSD, %) could not be determined due to the number of analyses (see Table 4.1.2-9).

Conclusion **GC-MS with selective ion monitoring can be used as confirmatory chromatographic technique in analytical method AR 144-97. It enables sensitive and safe detection of iprodione in plant products and fulfils the European requirements for confirmatory techniques as defined in 96/46/EC of 16 July 1996 and Guideline SANCO/825/00 rev.6 of 20 June 2000.**

Report: CA 4.1.2/9
Benz A.,Mackenroth C., 2004a
Validation of the analytical method No. 543/0: Determination of Iprodione BAS 610 F (Reg.No. 101 169) in plant matrices
2004/1010554
Guidelines: EPA 860.1340, EEC 96/46, SANCO/825/00 rev. 6 (20 June 2000), SANCO/3029/99 rev. 4 (11 July 2000)
GLP: yes
(certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Principle of the method

BAS 610 F is extracted with a mixture of acetone, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned against cyclohexane. The final determination of BAS 610 F is performed by HPLC-MS/MS.

Recovery findings

The average recovery in all matrices was between 70 and 110%.

Table 4.1.2-10: Validation data for analytical method 543/0 for the determination of BAS 610 F residues in food of plant origin

Matrix	Fortification level [mg/kg]	No of analyses	Average recovery [%]		RSD [%]	
Transition			330 m/z → 245 m/z	332 m/z → 247 m/z	330 m/z → 245 m/z	332 m/z → 247 m/z
Rice	0.01	5	92.8	91.7	2.2	3.5
	3.0	5	95.3	96.2	1.5	2.3
Tomato	0.01	5	91.3	92.2	3.0	1.6
	5.0	55	94.1	94.6	1.7	2.9
Lemon	0.01	5	90.9	94.0	5.5	5.0
	5.0	55	89.4	88.4	4.7	5.0
Oilseed rape	0.01	5	94.7	96.2	5.0	1.9
	0.50	55	93.2	92.1	4.4	4.2
Onion	0.01	5	93.1	93.6	2.3	3.4
	0.20	55	91.7	92.2	4.2	2.5
Broccoli	0.01	5	86.9	88.8	3.0	2.8
	0.10	55	93.7	89.5	1.3	2.2
Lettuce	0.01	5	83.5	87.7	1.8	2.3
	10	55	90.5	92.3	4.8	3.3
Strawberry	0.01	5	98.0	94.0	3.5	4.7
	15	55	93.9	93.9	5.4	3.7
Potato	0.01	5	88.5	88.3	3.7	1.7

Linearity	The detector response was found to be linear in the range of 0.025 to 0.50 ng/mL (R^2 > 0.999 for ion transition 330 m/z → 245 m/z).
Specificity	Since detection was carried out by MS/MS using two ion transitions, it can be considered that the determination of BAS 610 F was highly specific.
Limit of Quantitation	The method achieves a limit of quantitation (LOQ) of 0.01 mg/kg iprodione.
Repeatability	The relative standard deviations (RSD, %) for all matrices and fortification levels were below 20%. The detailed values are shown in Table 4.1.2-10.
Conclusion	**The results using BASF method No 543/0 to determine residues of iprodione in plant matrices proved that the method is suitable for the correct and accurate quantitation of residues in plant matrices.**

Report:	CA 4.1.2/10 Lehmann A., 2014a Validation of BASF Method No. L0180/01: Method for the determination of BAS 610 F (Reg.No. 101169) and its metabolites RP 32490 (Reg.No. 5079628), RP 30228 (Reg.No. 5079647), RP 32596 (Reg.No. 85831), RP 37176 (Reg.No. 5079612) and M610F007 (Reg.No. 5916256) in plant matrices 2011/1266284
Guidelines:	EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 6 (20 June 2000), EEC 96/46 (16.07.1996), SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the method

BAS 610 F and the metabolites RP 32490, RP 30228, RP 32596 and RP 37176 are extracted with a mixture of acetonitrile and water. An aliquot of the extract is centrifuged and measured directly. The metabolite M610F007 is extracted with methanol. An aliquot of the extract is centrifuged, diluted with water and measured directly.
The final determination of BAS 610 F and its metabolites RP 32490, RP 30228, RP 32596 and RP 37176 as well as M610F007 is performed by LC-MS/MS.

Recovery findings

The average recovery in all matrices was between 70 and 110%. The results are presented in Table 4.1.2-11 to Table 4.1.2-16.

Table 4.1.2-11: Validation data for analytical method L0180/01 for the determination of BAS 610 F residues in food of plant origin

Matrix	Fortification level [mg/kg]	No of analyses	Average recovery [%]		RSD [%]	
Transition			*330 m/z → 245 m/z	332 m/z → 247 m/z	330 m/z → 245 m/z	332 m/z → 247 m/z
Sunflower	0.01	5	90.4	88.6	1.9	3.9
	0.1	5	101.4	98.0	3.7	2.7
Wheat grain	0.01	5	98.2	93.0	8.2	5.5
	0.1	5	102.3	97.7	5.5	5.6
Cauliflower	0.01	5	94.9	97.0	4.9	4.0
	0.1	5	94.3	95.0	3.5	4.2
Carrot	0.01	5	95.3	92.8	3.9	4.0
	0.1	5	97.6	98.0	1.8	2.7
Chinese cabbage	0.01	5	97.7	95.4	2.1	3.5
	0.1	5	98.3	97.1	1.8	2.5
Broccoli	0.01	5	98.1	97.1	2.9	3.5
	0.1	5	93.8	95.0	4.3	3.8
Melon	0.01	5	99.0	98.2	2.1	2.0
	0.1	5	100.3	98.8	2.1	2.6
Tomato	0.01	5	95.0	98.9	6.3	4.2
	0.1	5	100.8	97.7	1.6	2.6

* used for quantitation

Table 4.1.2-12: Validation data for analytical method L0180/01 for the determination of RP 32490 residues in food of plant origin

Matrix	Fortification level [mg/kg]	No of analyses	Average recovery [%]		RSD [%]	
Transition			288 m/z → 245 m/z	290 m/z → 247 m/z	288 m/z → 245 m/z	290 m/z → 247 m/z
Sunflower	0.01	5	*99.0	99.0	1.7	4.3
	0.1	5	104.5	103.7	3.3	4.5
Wheat grain	0.01	5	100.9	98.1	8.1	9.4
	0.1	5	99.3	99.0	3.6	6.2
Cauliflower	0.01	5	101.6	98.6	6.2	6.3
	0.1	5	96.2	95.7	2.4	3.3
Carrot	0.01	5	95.5	97.6	3.8	2.5
	0.1	5	103.6	102.7	1.6	4.7
Chinese cabbage	0.01	5	87.8	96.4	5.7	3.2
	0.1	5	96.1	93.8	3.3	4.5
Broccoli	0.01	5	97.4	99.3	4.0	3.1
	0.1	5	89.9	90.1	10.3	6.4
Melon	0.01	5	86.6	90.4	4.3	3.5
	0.1	5	92.4	91.6	3.8	3.9
Tomato	0.01	5	83.7	86.3	1.9	3.5
	0.1	5	93.6	95.0	2.8	2.6

* used for quantitation

Table 4.1.2-13: Validation data for analytical method 180/01 for the determination of RP 30228 (Reg. No. 5079647) residues in food of plant origin

Matrix	Fortification level [mg/kg]	No of analyses	Average recovery [%]		RSD [%]	
Transition			*330 m/z → 101 m/z	330 m/z → 143 m/z	330 m/z → 101 m/z	330 m/z → 143 m/z
Sunflower	0.01	5	85.1	80.6	2.8	6.0
	0.1	5	98.6	100.6	3.7	5.6
Wheat grain	0.01	5	96.5	95.9	10.6	7.4
	0.1	5	99.6	97.3	5.1	2.7
Cauliflower	0.01	5	107.6	91.0	2.4	11.3
	0.1	5	98.0	100.9	4.5	3.9
Carrot	0.01	5	95.7	93.3	2.9	5.1
	0.1	5	97.3	98.2	2.2	2.5
Chinese cabbage	0.01	5	95.9	96.4	1.7	2.0
	0.1	5	98.8	97.7	1.6	3.7
Broccoli	0.01	5	99.4	101.3	5.1	5.8
	0.1	5	92.9	94.6	4.7	5.5
Melon	0.01	5	99.3	101.5	5.4	7.4
	0.1	5	101.8	100.0	1.1	4.4
Tomato	0.01	5	94.4	96.4	4.3	2.0
	0.1	5	97.3	98.7	0.9	3.3

* used for quantitation

Table 4.1.2-14: Validation data for analytical method L0180/01 for the determination of RP 32596 residues in food of plant origin

Matrix	Fortification level [mg/kg]	No of analyses	Average recovery [%]		RSD [%]	
Transition			*162 m/z → 127 m/z	162 m/z → 109 m/z	162 m/z → 127 m/z	162 m/z → 109 m/z
Sunflower	0.01	5	104.7	109.1	1.5	1.3
	0.1	5	105.2	104.8	2.1	0.8
Wheat grain	0.01	5	96.8	96.8	7.6	8.5
	0.1	5	101.5	101.8	4.2	3.2
Cauliflower	0.01	5	100.4	102.1	1.7	2.8
	0.1	5	98.3	95.8	2.2	3.5
Carrot	0.01	5	91.6	93.2	4.1	4.5
	0.1	5	91.8	91.3	3.7	3.6
Chinese cabbage	0.01	5	95.9	96.4	2.4	2.6
	0.1	5	97.6	97.2	5.3	3.7
Broccoli	0.01	5	100.6	99.5	2.9	3.3
	0.1	5	94.8	94.0	4.4	4.1
Melon	0.01	5	98.4	99.4	1.6	3.0
	0.1	5	99.8	99.3	3.0	1.6
Tomato	0.01	5	97.6	97.3	1.6	1.0
	0.1	5	96.3	99.1	2.3	1.4

* used for quantitation

Table 4.1.2-15: Validation data for analytical method L0180/01 for the determination of RP 37176 residues in food of plant origin

Sample matrix	Fortification level [mg/kg]	No of analyses	Average recovery [%]		RSD [%]	
Transition			*304 m/z → 219 m/z	306 m/z → 221 m/z	304 m/z → 219 m/z	306 m/z → 221 m/z
Sunflower	0.01	5	77.7	76.9	1.7	2.6
	0.1	5	104.1	101.0	2.7	3.3
Wheat grain	0.01	5	93.3	94.0	4.9	2.3
	0.1	5	97.5	97.2	2.2	0.7
Cauliflower	0.01	5	88.5	89.3	3.3	5.2
	0.1	5	95.7	95.3	2.0	2.6
Carrot	0.01	5	87.9	89.3	3.9	2.7
	0.1	5	109.8	108.4	2.1	1.9
Chinese cabbage	0.01	5	94.0	94.9	4.5	3.4
	0.1	5	105.0	105.0	3.0	3.3
Broccoli	0.01	5	96.0	99.1	4.2	4.8
	0.1	5	97.4	98.5	0.7	1.6
Melon	0.01	5	95.0	94.9	3.6	2.1
	0.1	5	98.6	96.8	1.5	2.3
Tomato	0.01	5	101.2	101.5	5.6	2.3
	0.1	5	104.2	106.0	0.4	2.0

* used for quantitation

Table 4.1.2-16: Validation data for analytical method L0180/01 for the determination of M610F007 residues in food of plant origin

Matrix	Fortification level [mg/kg]	No of analyses	Average recovery [%]		RSD [%]	
Transition			*291 m/z → 84 m/z	291 m/z → 130 m/z	291 m/z → 84 m/z	291 m/z → 130 m/z
Sunflower	0.01	5	90.2	91.8	2.4	3.5
	0.1	5	95.5	95.5	1.5	2.2
Wheat grain	0.01	5	85.1	82.7	1.7	2.6
	0.1	5	88.0	87.8	3.9	3.3
Cauliflower	0.01	5	96.6	96.6	1.4	1.0
	0.1	5	89.6	90.2	2.6	2.2
Carrot	0.01	5	101.0	96.9	3.8	3.2
	0.1	5	95.4	98.6	3.5	2.9
Chinese cabbage	0.01	5	101.4	94.0	6.8	5.0
	0.1	5	99.7	95.5	2.8	2.9
Broccoli	0.01	5	100.6	96.1	2.4	1.9
	0.1	5	94.1	99.0	1.8	0.7
Melon	0.01	5	98.6	94.5	2.2	6.0
	0.1	5	98.9	98.9	2.3	3.6
Tomato	0.01	5	96.6	98.2	2.0	1.0
	0.1	5	95.5	97.0	1.0	2.4

* used for quantitation

Linearity	Good linearity (r > 0.99) was observed in the range of 0.1 ng/mL and accordingly 0.05 ng/mL to 2.5 ng/mL for the two mass transitions of BAS 610 F-, RP 32490-, RP 30228-, RP 32596-, RP 37176-mix standard solutions and M610F007 standard solution.
Specificity	Since detection was carried out by LC-MS/MS using two different ion transitions, it can be considered that the determination of BAS 610 F and its metabolites RP 32490, RP 30228, RP 32596 and RP 37176 as well as M610F007 was highly specific. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions of each analyte.
Limit of Quantitation	The method achieves a limit of quantitation (LOQ) of 0.01 mg/kg for all analytes.
Repeatability	The relative standard deviations (RSD, %) for all matrices, all analytes and all fortification levels were below 20%. The detailed values are shown in Table 4.1.2-11 to Table 4.1.2-16.
Reproducibility	Reproducibility of the method was not determined within this validation study.
Conclusion	**BASF analytical method L0180/01 is suitable to determine residues of BAS 610 F and its metabolites RP 32490, RP 30228, RP 32596, RP 37176 and M610F007 in plant matrices (sunflower, wheat grain, cauliflower, carrot, Chinese cabbage, broccoli, melon, tomato) at a limit of quantitation of 0.01 mg/kg. It could be demonstrated that the method fulfills the requirements with regard to specificity, repeatability, limit of quantitation and recoveries.**

Animal

Concerning animal matrices a variety of methods exists. An overview is presented here for the reviewers' convenience.

Table 4.1.2-17: Summary table of analytical methods submitted for the quantitation of iprodione and its metabolites in animal matrices

Method	LOQ (mg/kg)	Matrix	Analyte(s)	Detection
Craig, 1982 (C025664)	0.1	Kidney, muscle, fat, liver	Iprodione RP32490	GC-ECD
Craig, 1982 (C025673)	0.01	milk	Iprodione RP32490	GC-ECD
Craig, 1982 (C025672)	0.01	milk	RP36114	GC-ECD
Gemma, 1983 (C025674)	0.01 (egg) 0.1 (muscle, fat)	Egg, muscle, fat	RP32490	GC-ECD
AR 230-99 (CA 4.1.2/11; C021239)	0.01	Milk	Iprodione RP32490	GC-ECD
AR 230-99 (CA 4.1.2/12; R014639)	0.005 (milk)	Milk, egg, meat, liver	RP32490	GC-ECD (Confirmatory: GC-MS)
AR 230-99 (CA 4.1.2/13; R014654; ILV)	0.005 (milk) 0.10 (meat)	Milk, meat	RP32490	GC-ECD

The following methods for data generation have so far only been submitted on national level and were not peer-reviewed. Summaries are therefore included here for completeness and have not been mentioned in the application previously:

Report: CA 4.1.2/11
Guillet M.,Yslan F., 1998b
Iprodione and its metabolites: Replacement of Benzene by Toluene in the analytical methods for their determination in products of animal origin, exemplified with milk
C021239
Guidelines: EEC 96/46
GLP: yes
(certified by Groupe Interministeriel des Produits Chimiques, France)

The objective of the study was to verify that toluene can successfully replace benzene in the methods for analysis of iprodione and its metabolites in products of animal origin.

Principle of the method

The samples are extracted using acetone and water. After evaporation and liquid-liquid partition with acetonitrile and n-hexane, the extracts are hydrolyzed into 3,5 dichloroaniline and distilled. The aniline is then extracted by liquid-liquid partition with toluene and then derivatized using heptafluorobutyric anhydride to form the 3,5-dichloroaniline-1-heptafluorobutyranilide (RPA414679). The final derivative is cleaned up by Florisil column chromatography and the final determination is made by gas chromatography on a semi-capillary column using electron capture detection (ECD) and quantitation by external standardization.

Recovery findings

The average recovery in milk was between 70 and 110% for both iprodione and RP 32490.

Table 4.1.2-18: Validation data for analytical methods for the determination of BAS 610 F residues in milk

Sample matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No of analyses
Milk	0.01	85	4	5

Table 4.1.2-19: Validation data for analytical methods for the determination of RP 32490 residues in milk

Sample matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No of analyses
Milk	0.01	82	10	5

Linearity	The detector response was found to be linear in the range of 10 to 50 µg/L.
Specificity	Interference due to substrate was <10% LOQ.
Limit of Quantitation	The method achieves a limit of quantitation (LOQ) of 0.01 mg/kg.
Repeatability	The relative standard deviations (RSD, %) were below 20% for both analytes. The detailed values are shown in Table 4.1.2-17 and Table 4.1.2-18.
Conclusion	**The use of toluene instead of benzene was found to have no incidence in the analysis of iprodione and its metabolite RP 32490 in milk. Consequently, the acceptability of the replacement of benzene by toluene was verified. The extrapolation for the other methods allowing the analysis of iprodione and its metabolite in products of animal origin is allowed.**

Report: CA 4.1.2/12
Kieken J.-L. et al., 1999b
Iprodione and its metabolites: Analytical method for the determination of residues in products of animal origin
R014639
Guidelines: EEC 96/46
GLP: yes
(certified by Groupe Interministeriel des Produits Chimiques, France)

The objective of the study was to complete the validation of study C021239 summarized above.

Principle of the method

The samples are extracted using acetone and water. After evaporation and liquid-liquid partition with acetonitrile and n-hexane, the extracts are hydrolyzed into 3,5 dichloroaniline and distilled. The aniline is then extracted by liquid-liquid partition with toluene and then derivatized using heptafluorobutyric anhydride to form the 3,5-dichloroaniline-1-heptafluorobutyranilide (RPA414679). The final derivative is cleaned up by Florisil column chromatography and the final determination is made by gas chromatography on a semi-capillary column using electron capture detection (ECD) and quantitation by external standardization.

Recovery findings

The average recoveries were between 70 and 110% for all matrices and all fortification levels.

Table 4.1.2-20: Validation data for analytical method AR 230-99 for the determination of RP 32490 residues in products of animal origin

Sample matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Milk	0.005 0.050	96 86	3 3	5 5
Egg	0.10	90	2	5
Meat	0.10	86	5	5
Liver	0.10	92	4	5

Linearity The detector response was found to be linear in the range of 5 to 100 µg/L.

Specificity Interferences due to reagents were estimated to be <10% LOQ. Interferences due to substrates were at most <20% of the (highest) fortification level.
GC-MS technique can be used as qualitative confirmatory chromatographic method for the identification of the compound.

Limit of Quantitation	The method achieves a limit of quantitation (LOQ) of 0.005 mg/kg for milk and was not reported for eggs, meat and liver.
Repeatability	The relative standard deviations (RSD, %) for all matrices were below 20%. The detailed values are shown in Table 4.1.2-19.
Reproducibility	The results of the independent laboratory validation are presented below (DocID R014654).
Conclusion	**The method proved to be suitable for analysis of animal tissues, milk and eggs.**

Report:	CA 4.1.2/13 Fuchsbichler G., 2000c Independent laboratory validation of analytical method AR 230-99 for the determination of RP 32490 (Iprodione metabolite) in animal products: Beef meat and milk R014654
Guidelines:	EEC 96/46, EU Guideline 8064/VI/97 rev. 4 15.12.1998
GLP:	yes (certified by Bayerisches Staatsministerium fuer Arbeit, Familie und Sozialordnung, Muenchen)

Principle of the method

Residues are extracted from the sample material with acetone/water. The extract is evaporated to an aqueous residue, cleaned up with n-hexane and partitioned into acetonitrile. The residue is then hydrolyzed into 3,5-dichloroaniline which is distilled, partitioned into toluene and reacted with heptafluorobutyric anhydride. The obtained 3,5-dichloroheptafluorobutyranilide (RPA414679) is finally cleaned up on a Florisil column. The analysis is carried out using gas chromatography with electron capture detection (GC/ECD), quantitation being done by external standardization.

Recovery findings

The average recovery was between 70 and 110% for both matrices and all fortification levels.

Table 4.1.2-21: Validation data for analytical method AR 230-99 for the determination of RP 32490 residues in products of animal origin

Sample matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No of analyses
Meat	0.10 1.0	95 88	4.7 7.9	5
Milk	0.005 0.05	83 78	12.1 8.8	5

Linearity The detector response was found to be linear in the range of 0.005 to 0.02 µg/mL (R^2> 0.99).

Specificity The level of interferences measured in the control samples was <30% LOQ.

Limit of Quantitation	The method achieves a limit of quantitation (LOQ) of 0.005 mg/kg for milk and 0.10 mg/kg for meat.
Repeatability	The relative standard deviations (RSD, %) for both matrices were below 20%. The detailed values are shown in Table 4.1.2-20.
Reproducibility	The method was successfully independently validated.
Conclusion	**Analytical method AR230-99 therefore was successfully independently validated. It enables sensitive and safe determination of RP 32490 in animal products (beef meat and milk).**

Additionally a new method is under development covering both analytes with 3,5-dichloroaniline-containing and 4-hydroxy-3,5-dichloroaniline-containing moieties at an LOQ of 0.01 mg/kg in relevant animal matrices (BASF DOC ID 2013/1311894). However, this should be considered as supplemental information since no additional data in animal matrices for registration purposes has been generated or is currently planned to be generated.

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

Methods for concentration control are reported, where necessary, along with the respective ecotoxicological studies (including 2013/1311888 as mentioned in the application previously).

(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

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

Based on the proposed residue definitions for monitoring as summarized in Document N, chapter 7.3 and discussed in CA 6.7 for products of plant and animal origin the following methods are valid for post-approval control and monitoring purposes of Iprodione and/or its metabolites if necessary in these compartments.

The proposed residue definition for enforcement is:

Plant: Iprodione
Animal: RP 32490

The provided data is also designed to also cover the data requested by EFSA in 2013 (Reasoned opinion, 2013;11(10):3438).

Plant

Validation data as well as results from an independent laboratory validation are presented using the multi residue method QuEChERs for the parent compound.

Sufficient extraction efficiency was demonstrated for the most representative matrix carrot (root and forage) in a recent metabolism study (CA 6.2.1/1) covering matrices with high water/acid content. No testing was possible for dry and oily commodities due to no available samples (hot or cold) for testing. However, the respective metabolism study (refer to CA 6.2, BAS Doc ID C023023) besides acetone also included acetonitrile in the extraction scheme yielding high extractable residue values.

Report: CA 4.2/1
Diamaduros B.K.,Andrews R.S., 2014a
Validation of BASF analytical method D1309: Analytical method for the determination of the residues of BAS 610 F in plant matrices using QuEChERS at a loq of 0.01 mg/kg using LC-MS/MS
2013/1311893

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 (11 July 2000), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07

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

Principle of the method Residues of BAS 610 F are extracted from plant matrices with acetonitrile. A mixture of salts ($MgSO_4$, NaCl, trisodium citrate dihydrate, and disodium hydrogencitrate sesquihydrate) was added to separate the aqueous and organic layers. The sample was partitioned by shaking and then centrifuged. An aliquot of the resulting organic extract was treated with $MgSO_4$ and dispersive SPE clean-up. The extract was then centrifuged and the supernatant was diluted in 0.1% formic acid in acetonitrile/water. The residues were determined by HPLC-MS/MS monitoring two ion mass transitions for quantitation and confirmation.

Recovery findings Mean recoveries for all plant matrices (lettuce, barley grain, soya bean seed, dry bean and grape) were between 70 and 120% per fortification level for both ion transitions.

Table 4.2-1: Validation data for analytical method QuEChERS (D1309) for the determination of BAS 610 F residues in from plant matrices

Test substance	Matrix	Fortification level (mg/kg)	No of tests	Average recovery [%]		Relative standard deviation [%]	
Transition				330 m/z → 245 m/z*	332 m/z → 247 m/z	330 m/z → 245 m/z*	332 m/z → 247 m/z
BAS 610 F	Lettuce	0.01	5	106	105	3	4
		0.1	5	102	99	3	4
	Barley grain	0.01	5	109	109	3	11
		0.1	5	113	117	6	4
	Soya bean seed (dried)	0.01	5	93	92	4	8
		0.1	5	91	88	2	2
	Dry bean	0.01	5	103	103	13	7
		0.1	5	98	96	5	11
	Grape	0.01	5	97	100	6	4
		0.1	5	99	98	8	4

* proposed for quantitation

Linearity	Acceptable linearity was observed for the standard range and the two mass transitions tested for each analyte: The method-detector response was linear over the 0.1-2 ng/mL range ($R^2 \geq 0.9887$) for dried or low-moisture content matrices and also linear over the 0.25-5 ng/mL range ($R^2 \geq 0.9887$) for fresh fruits and vegetable matrices.
Specificity	LC-MS/MS analysis was performed at two ion transitions; therefore no confirmatory method is necessary. No interfering peaks were found at the retention times (<30% of LOQ).
Limit of Quantitation	The limit of quantitation (LOQ) of the method is 0.01 mg/kg.
Repeatability	The relative standard deviations (RSD, %) for all matrices were below 20%. The detailed values are shown in Table 4.2-1.
Reproducibility	Reproducibility of the method was not determined within this validation study.
Conclusion	The analytical BASF method D1309 is suitable for determining residues of BAS 610 F in lettuce, barley grain, dry bean and grape down to a limit of quantitation of 0.01 mg/kg. It could be demonstrated that the method fulfills the requirements with regard to specificity, repeatability, limit of quantitation and recoveries.

Report: CA 4.2/2
Bianca C.M., 2014a
Independent laboratory validation of BASF analytical method D1309: Determination of the residues of BAS 610 F in plant matrices using QuEChERS at a LOQ of 0.01 mg/kg using LC/MS/MS
2013/1311889

Guidelines: EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 8.1 (16 November 2010), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07

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

Principle of the method Residues of iprodione (BAS 610 F) were extracted from soya bean seed, kidney bean, lettuce, barley grain, and grape using acetonitrile as extraction solvent; if dry matrix addition of water was necessary to hydrate. The extract was partitioned using the addition of the QuEChERS method of salt containing magnesium sulfate salt, sodium chloride, sodium citrate dibasic sesquihydrate, and sodium citrate tribasic, then centrifuged for separation of organic and aqueous layers. An aliquot was taken and cleaned up using magnesium sulfate PSA portion to clean up the sample. The samples were centrifuged and diluted properly with (10:90, v/v) acetonitrile and water with 0.1% formic acid. The residues were determined using HPLC-MS/MS.

Recovery findings Mean recoveries for all plant matrices (lettuce, barley grain, soya bean seed, kidney bean and grape) were between 70 and 120% per fortification level for both ion transitions.

Table 4.2-2: Independent laboratory validation data for analytical method QuEChERS (D1309) for the determination of BAS 610 F residues in from plant matrices

Test substance	Matrix	Fortification level (mg/kg)	No of tests	Average recovery [%]		Relative standard deviation [%]	
Transition				330 m/z → 245 m/z*	332 m/z → 247 m/z	330 m/z → 245 m/z*	332 m/z → 247 m/z
BAS 610 F	Lettuce	0.01	5	102	110	2.7	4.1
		0.1	5	102	103	5.3	2.2
	Barley grain	0.01	5	82	85	3.5	6.1
		0.1	5	107	113	6.9	3.0
	Soya bean seed	0.01	5	94	92	7.8	10.3
		0.1	5	110	112	3.3	8.7
	Kidney bean	0.01	5	111	112	3.0	5.2
		0.1	5	108	106	2.3	6.5
	Grape	0.01	5	84	90	7.1	7.5
		0.1	5	81	81	11.5	8.2

* proposed for quantitation

Linearity	Good linearity was observed in the range tested (0.1-5.0 ng/mL) with correlation coefficients R^2>0.999 for barley, shown as representative chromatograms in the report.
Specificity	LC-MS/MS analysis was performed at two ion transitions; therefore no confirmatory method is necessary. No interfering peaks were found at the retention times (<30% of LOQ).
Limit of Quantitation	The limit of quantitation (LOQ) of the method is 0.01 mg/kg.
Repeatability	The relative standard deviations (RSD, %) for all matrices were below 20%. The detailed values are shown in Table 4.2-2.
Reproducibility	The independent laboratory validation was successfully completed for the analysis of BAS 610 F in all plant matrices tested.
Conclusion	The method is suitable for enforcement purposes.

Animal

Validation data as well as results from an independent laboratory validation are presented for RP 32490.
Comparable extraction solvents, i.e. acetone as in the metabolism studies (please refer to CA 6.2) are used in these methods. Additionally, no residues above the LOQ are expected in animal matrices based on the feed burden calculation discussed in Chapter 6.7.

Report:	CA 4.2/3 Kuhn T., 2014b Development and validation of an analytical method for the determination of the Iprodione metabolite Reg. No. 5079628 (formerly RP32490) in foodstuffs of animal origin 2013/1311890
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Principle of the method The iprodione metabolite Reg. No 5079628 is extracted from animal materials with acetone. An aliquot of the acetone is concentrated to dryness and re-constituted in acetonitrile/water (50/50, v/v) and 0.1% formic acid. Final determination of the analyte is achieved for all matrices by LC-MS/MS, using MRM transitions for quantitation and confirmation.

Recovery findings The average recoveries for all matrices were between 70 and 110% per fortification level for both ion transitions.

Table 4.2-3: Validation data for analytical methods for the determination of RP 32490 residues in products of animal origin

Test substance	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)		Relative standard deviation (%)	
Transition				288 m/z → 245* m/z	290 m/z → 247 m/z	288 m/z → 245* m/z	290 m/z → 247 m/z
RP 32490 (Reg. No 5079628)	Milk	0.01	5	93	89	5	9
		0.1	5	96	96	3	6
	Egg	0.01	5	94	93	5	4
		0.1	5	98	97	2	4
	Meat	0.01	5	101	100	2	4
		0.1	5	103	103	4	5
	Liver	0.01	5	94	86	6	7
		0.1	5	97	96	3	3
	Kidney	0.01	5	90	88	4	10
		0.1	5	94	95	4	5
	Fat	0.01	5	75	79	12	15
		0.1	5	96	99	6	7

* proposed for quantitation

The stock solution of the iprodione metabolite Reg. No 5079628 prepared in acetonitrile was stored refrigerated for up to 34 days prior to dilution. This diluted solution was then examined by LC-MS/MS and the obtained peak areas were used to demonstrate stability of the analyte after refrigerated storage compared to peak areas of the freshly prepared and diluted stock solutions.
Consistent recovery results demonstrate stability of fortification solutions prepared in acetonitrile for at least 13 days. Stability of extracts fortified at LOQ level (0.01 mg/kg) was demonstrated for all animal matrices when stored refrigerated with recovery results in the range of 68 to 109%.

Linearity The detector response was found to be linear in the range of 0.15-6.0 ng/mL with correlation coefficients $R^2 \geq 0.99$.

Specificity LC-MS/MS analysis was performed at two ion transitions; therefore no confirmatory method is necessary.
Residues in all blank control samples were below 30% LOQ, showing that no significant signal interference was caused by the analytical method.

Limit of Quantitation	The limit of quantitation (LOQ) of the method is 0.01 mg/kg.
Repeatability	The relative standard deviations (RSD, %) for all matrices were below 20%. The detailed values are shown in Table 4.2-3.
Reproducibility	The results of the independent laboratory validation are shown below (DocID 2013/1311891).
Conclusion	The analytical method was successfully validated for animal matrices.

Report: CA 4.2/4
Benotti M.J., 2014b
Independent laboratory validation of analytical method for the determination of the Iprodione metabolite Reg.No. 5079628 (formerly RP32490) in foodstuffs of animal origin
2013/1311891

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), EPA 860.1340, OECD-ENV/JM/MONO/(2007)17 (OECD No. 39), EPA PR Notice 96-1, EPA PR Notice 2011-3

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

Principle of the method The iprodione metabolite Reg. No 5079628 (RP 32490) is extracted from animal materials with acetone. An aliquot of the acetone is concentrated to dryness and re-constituted in acetonitrile/water (1/1, v/v) and 0.1% formic acid. Final determination of the analyte is achieved for all matrices by LC-MS/MS, using MRM transitions for quantitation and confirmation.

Recovery findings The average recoveries for all matrices were between 70 and 110% per fortification level for both ion transitions.

Table 4.2-4: Independent laboratory validation data for analytical methods for the determination of RP 32490 residues in products of animal origin

Test substance	Matrix	Fortification level (mg/kg)	No of tests	Average recovery (%)		Relative standard deviation (%)	
Transition				288 m/z → 245* m/z	290 m/z → 247 m/z	288 m/z → 245* m/z	290 m/z → 247 m/z
RP 32490 (Reg. No 5079628)	Milk	0.01	5	72	72	8	10
		0.1	5	70	73	2	1
	Egg	0.01	5	77	77	6	6
		0.1	5	71	74	2	4
	Meat	0.01	5	76	71	13	16
		0.1	5	70	73	4	6
	Liver	0.01	5	78	73	13	10
		0.1	5	70	72	3	2
	Kidney	0.01	5	76	72	5	3
		0.1	5	70	70	4	4
	Fat	0.01	5	78	71	7	15
		0.1	5	79	83	6	5

* proposed for quantitation

Linearity	The detector response was found to be linear in the range of 0.15-6.0 ng/mL with correlation coefficients $R^2 \geq 0.99$.
Specificity	LC-MS/MS analysis was performed at two ion transitions; therefore no confirmatory method is necessary. Residues in all blank control samples were below 30% LOQ, showing that no significant signal interference was caused by the analytical method.
Limit of Quantitation	The limit of quantitation (LOQ) of the method is 0.01 mg/kg.
Repeatability	The relative standard deviations (RSD, %) for all animal matrices were below 20%. The detailed values are shown in Table 4.2-4.
Reproducibility	The results of the independent laboratory validation confirmed the good validation results.
Conclusion	The analytical method was successfully independently validated for animal matrices.

(b) Methods for the analysis in soil and water

Based on the proposed residue definitions for monitoring in soil, ground water, surface water and sediment as described in Document N, chapter 8.5 the following methods are valid for post-approval control and monitoring purposes of Iprodione in these compartments.

Residue definition for monitoring purposes:

Soil: Iprodione, RP 30228, RP 36221 and dichloroaniline
Sediment: Iprodione, RP 30228, RP 35606
Water: Iprodione, RP 30228, RP 35606
Air: Iprodione

Soil

Validation data for the analysis of parent, RP30228 as well as RP35606 in soil are presented in CA 4.1.2/2 (2013/1400376) while RP 36221 and dichloroaniline are included in CA 4.1.2/1.
Since the extraction solvent contains a significant amount of water the method is also suitable for sediment if needed.
Similar extraction solvent mixtures consisting of acetonitrile and water as in the recent metabolism study (CA 7.1) are being used in the presented residue method.

Water

Validation data for parent, RP30228 as well as RP35606 in water are presented in CA 4.1.2/4 (2013/1311332).
Additionally results for the same analytes from an independent laboratory validation are given below.

Due to the combination of all three analytes in the same validation (2013/1400376) the independent lab validation study planned in the application for the individual method are not needed anymore (i.e. 2013/7002481).

Report:	CA 4.2/5 Michener P., 2014a Independent laboratory validation of PTRL Europe ID P 2999G: Determination of Iprodione (BAS 610 F), Iprodione degradate/intermediate Reg.No. 5079626 (formerly RP35606) and Iprodione Isomer RP30228 in surface and drinking water 2013/7002482
Guidelines:	EPA 850.6100, SANCO/825/00 rev. 8.1 (16 November 2010), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07
GLP:	yes (certified by United States Environmental Protection Agency)

Principle of the methods

For the analysis of Iprodione and its intermediate RP35606 (Reg.No. 5079626), a 60 mL portion of each water matrix was adjusted to pH 5 with diluted formic acid. A 1 mL aliquot of the pH 5 sample was directly injected into the liquid chromatograph and analysed by LC-MS/MS for Iprodione and its intermediate RP35606.

For the extraction and analysis of Iprodione and its isomer RP30228 (Reg.No. 5079647) a second method was used, using C^{18}–based solid-phase extraction (SPE) of 100-mL water samples, followed by elution of the analytes with 2.5 mL acetonitrile and subsequent LC-MS/MS analysis of the enriched water extracts.

Recovery findings

The methods proved to be suitable to determine all three analytes in water, with an LOQ of 0.03 µg/L. Validation experiments were conducted in surface and drinking water. All average recovery values were between 70% and 120%, except the recoveries of Iprodione (Reg.No. 101169) in surface water at the fortification level 0.03 µg/L for both mass transitions, which were with 123.4% and 126.1% out of this range. The detailed results are given in Table 4.2-5 and Table 4.2-6.

Table 4.2-5: Summary of Recoveries of Iprodione and the Iprodione degradate / intermediate Reg.No. 5079626 (formerly RP35606) in tap water and surface water with the DI-LC-MS/MS method

Analyte	m/z	Matrix	Replicates	Fortification level [µg/L]	Mean recovery [%]	RSD [%]
Iprodione Reg.No. 101169	**330→245**	tap water	5	0.03	101.8	6.8
				0.3	110.3	3.8
		surface water	5	0.03	94.3	8.4
				0.3	101.4	8.4
	332→247	tap water	5	0.03	102.8	6.2
				0.3	109.7	2.9
		surface water	5	0.03	100.5	6.6
				0.3	102.1	4.9
Reg.No. 5079626	**348→263**	tap water	5	0.03	106.8	6.4
				0.3	102.9	0.8
		surface water	5	0.03	89.5	3.2
				0.3	99.2	3.0
	348→76	tap water	5	0.03	88.9	3.8
				0.3	88.1	6.4
		surface water	5	0.03	90.7	6.3
				0.3	100.0	1.6

Table 4.2-6: Summary of Recoveries of Iprodione and the Iprodione isomer RP30228 (Reg.No. 5079647) in tap water and surface water with the SPE-LC-MS/MS method

Analyte	m/z	Matrix	Replicates	Fortification level [µg/L]	Mean recovery [%]	RSD [%]
Iprodione Reg.No. 101169	**330→245**	tap water	5	0.03	86.3	15.0
				0.3	82.6	8.1
		surface water	5	0.03	123.4	11.5
				0.3	115.2	5.5
	332→247	tap water	5	0.03	90.9	12.2
				0.3	84.8	8.1
		surface water	5	0.03	126.1	7.7
				0.3	117.1	3.0
RP30228 Reg.No. 5079647	**330→101**	tap water	5	0.03	97.1	17.6
				0.3	83.2	11.1
		surface water	5	0.03	104.4	10.4
				0.3	103.5	5.1
	332→101	tap water	5	0.03	99.5	16.8
				0.3	88.0	12.8
		surface water	5	0.03	106.8	6.4
				0.3	102.9	0.8

Linearity	Good linearity ($r \geq 0.99$) was observed for the two mass transitions in the two different water types (tap water and surface water).
Specificity	LC-MS/MS using two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required (*SANCO/825/00 rev.8, 16/11/2010*). There were no known interferences from plant components or from reagents, solvents and glassware used.
Limit of Quantitation	The method had a limit of quantitation (LOQ) of 0.03 µg/L for each analyte.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 4.2-5 and Table 4.2-6.
Reproducibility	In context of this ILV study, the reproducibility of this analytical method was estimated. As can be seen from the results, a high reproducibility was determined.
Conclusion	The report describes the independent validation of the analytical method "Determination of Iprodione (BAS 610 F, Reg. No. 101169), Iprodione Degradate/Intermediate Reg. No. 5079626 (formerly RP35606) and Irpodione Isomer RP30228 (Reg. No. 5079647) in Surface and Drinking Water. The results confirm that this analytical method is suitable to determine Iprodione and its intermediate and its isomer in water.

(c) Methods for the analysis in air

A validated method for Iprodione is presented in CA 4.1.2 (R014656) according to the proposed residue definitions for monitoring (i.e. parent only) in air as described in Document N, chapter 8.5.

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

Since Iprodione is not classified as toxic or very toxic, methods of analysis for parent or metabolites in human body tissues or fluids are not required.

Iprodione

DOCUMENT M-CA, Section 5

TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

Compiled by:

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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

Introduction

Studies relevant for toxicology

New studies were conducted to support the evaluation of toxicology properties of iprodione. Those studies, together with older studies relevant for the evaluation were included in the Application submitted in October 2013.
In the meantime further studies conducted between 1974 and 2002 have been considered relevant and are included in the present dossier. They are listed in Table 5-1 below for easy reference.
In addition, some references were found relevant in scientific literature database. The list of relevant hits from this literature search was not available in October 2013 and hence has not been listed in the Application. Those hits are included in the present dossier and listed as well in Table 5-1 below for easy reference.

Table 5-1: Overview of studies / reports / documents which were not listed in Application and included in the present dossier

DocID	Study Description	Year	Comment	Reference
B003580	Histopathology peer review and pathology working group review of ovarian lesions in CD-1 mice from a 24-month oral toxicity/oncogenicity study of iprodione with a 12-month interim sacrifice.	2001	Important for toxicological relevance assessment	Chapter MCA 5.5
C022570	Study Amendment: potential tumourigenic and toxic effects in prolonged dietary administration to rats	1999	Important for toxicological relevance assessment	Chapter MCA 5.5
C022572	Interstitial cell tumours of testis, reported in the carcinogenicity study of rats with lprodione (26019 RP), Huntingdon Life Sciences Report No. RNP346/920808 Review of interstitial cell adenomas in historical control data	1998	Important for toxicological relevance assessment	Chapter MCA 5.5
C022575	Iprodione - chronic rat study RNP 346/920808 Historical Control Data - Two year carcinogenicity studies started in 1989. Review performed February 1996	1996	Important for toxicological relevance assessment	Chapter MCA 5.5
C022990	30.228 R.P.: Acute toxicity and local tolerance	1976	completes the toxicological database of a metabolite - uncritical	Chapter MCA 5.8.1
C043629	Reverse mutation assay by the AMES test - RP30228	1984	Important for toxicological relevance assessment	Chapter MCA 5.8.1
C043621	13-week toxicity study by oral route (dietary admixture) in rats - RP30228	1993	Important for toxicological relevance assessment	Chapter MCA 5.8.1
C043657	Intermediates in the manufacture of 26019 R.P.: 25040 R.P. and 32247 R.P. - Toxicity and local tolerance	1974	Completes the toxicological database of a metabolite - uncritical	Chapter MCA 5.8.1
1986/1002731 1987/1002734 1988/1003296 1990/1004192 1990/1004193 1992/1005217 1994/1005350 1997/1008101 2000/1024057	Reference list for literature on 3,5-dichloroaniline	1986 - 2000	Literature Search Hits	Chapter MCA 5.8.1
B003653	Iprodione - Quantification of Iprodione and metabolites in the plasma and testes of the rat following a single oral administration of (14C)-Iprodione	1998	completes the toxicological database of a metabolite - uncritical	Chapter MCA 5.8.2
R014684	Iprodione - Hepatocellular proliferation and toxicity in the CD-1 mouse following 90 days of continual dietary administration	2000	Important for toxicological relevance assessment	Chapter MCA 5.8.2

DocID	Study Description	Year	Comment	Reference
R014678	Human androgen receptor binding assay with the fungicide Iprodione and 7 mammalian metabolites	1999	completes the toxicological database of a Iprodione/metabolite - uncritical	Chapter MCA 5.8.2
B003656	Iprodione - Hormonal measurements in adult male Sprague Dawley rats following administration of Iprodione by gavage	1998	Important for toxicological relevance assessment	Chapter MCA 5.8.2
C019327	Iprodione - Hormonal measurements in adult male Sprague Dawley rats following a single administration of Iprodione by gavage	2001	Important for toxicological relevance assessment	Chapter MCA 5.8.2
C024675	Iprodione - Hormonal measurements following a 14-day treatment period with Iprodione by gavage in the rat	2002	Important for toxicological relevance assessment	Chapter MCA 5.8.2
C025273	Iprodione - Measurement of leydig cell proliferation following a 14-day treatment period with Iprodione by gavage in the rat	2002	Important for toxicological relevance assessment	Chapter MCA 5.8.2
R014642	Cell proliferation in rat testes	1996	Important for toxicological relevance assessment	Chapter MCA 5.8.2
2008/1101757 2009/1130622 2004/1036097 2013/1347909 1999/1013778 2013/1347912 2006/1050814 2008/1101756 1999/1013777 2001/1031823 2010/1231472 2005/1043040 2006/1050813 2013/1347911 2011/1295091 2013/1371960	Reference list for literature on Iprodione	2008 - 2013	Literature Search Hits	Chapter MCA 5.8.3
2013/1168537 2014/1095062 2014/1095063 2014/1095066 2014/1095067 2014/1095075 2014/1095068 2014/1095065 2014/1092554	Analytical reports, were analytics are still on-going will be provided after submission	2013 - 2014	Analytical Reports	Reference List, only

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

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

Studies presented in the original Annex II Dossier (1995) or the update of the Annex II Dossier (1997): The earliest study (non GLP) to use radiolabelled iprodione (^{14}C-uniformly labelled phenyl ring) investigated the fate of (^{14}C-)-iprodione in the rat after oral administration by gavage (BASF DocID C022928). The (^{14}C-)-iprodione, diluted with non-radiolabelled iprodione and formulated as a suspension in 10% aqueous gum arabic, was administered to 2 male and 2 female rats (Charles River, France) as a single oral dose, at a nominal concentration of 100 mg/kg. A second group of rats (20) were dosed with non-radiolabelled iprodione at 200 mg/kg (nominally) as a single, oral dose. These rats were housed in similar conditions as the first group except that exhaled air was not trapped. Urine and feces from both dose groups were pooled per sex and per time period.

The following study (GLP) investigating the absorption, distribution, metabolism and excretion of (^{14}C)-iprodione following oral administration has been carried out by Life Science Research (LSR) (BASF DocID C021582), at the EPA's request. iprodione uniformly labelled with ^{14}C in the phenyl ring was diluted with non-radiolabelled iprodione, formulated as a suspension in 0.5% w/v aqueous carboxymethylcellulose and administered at a nominal dose level of either 50 mg or 900 mg (^{14}C)-iprodione/kg, by oral gavage to Charles River UK CD rats of both sexes. In addition to those animals extra male and female rats were dosed at a nominal 50 mg/kg and 900 mg/kg to provide blood pharmacokinetics data.

An additional study (GLP) (BASF DocID C022615) was conducted at the request of the EPA to upgrade the existing rat metabolism study by identifying a greater proportion of the radioactive metabolites found in the excreta. The absorption metabolism and excretion of iprodione in the rat were investigated following single oral administration at a nominal dose level of 50 mg/kg body weight, in accordance with international codes of Good Laboratory Practice and FIFRA: Office of Pesticides Programmes, Agrochemical Assessment Guidelines, Subdivision F,85-1, (EPA, Nov 1984).

The biokinetics and metabolism of iprodione uniformly labelled with ^{14}C in the phenyl ring was studied in rats (3 males and 3 females) of the Hairless strain obtained from IFFA-CREDO (France), following a topical (skin) application at 200 mg/kg. Twenty-four hours following application of the compound, the rats were killed by exsanguination and tissues (blood, liver, kidneys, gastrointestinal tract, skin-area of application and remaining skin) removed for analysis. (BASF DocID C022987).

A summary of the elimination, tissue retention and overall recovery data is given in **Table 5.1.1-1.**

Table 5.1.1-1: Excretion of total radioactivity and tissue residues following administration of (^{14}C)-iprodione

Study ref.	Dose (mg/kg) nominal	Route (& No. of rats)	Live phase (hours)	% dose eliminated in			Total tissue Residue (%Dose)[b]	Overall Recovery (%)
				Urine[a]	Faeces	CO_2		
Laurent et al., 1976 BASF DocID C022928	100	Oral (4)	96	62	36	<0.01	0.83	>99
Hallifax et al., 1989 BASF DocID C021582	50	Oral (>10)	168	61	32	<0.02	0.2	93
	50[c]	Oral (>10)	168	71	24	<0.02	0.35	96
	900	Oral (>10)	168	45	54	<0.02	0.1	99
[redacted], 1993 BASF DocID B003653	50	Oral (20)	168	40	53	-[d]	-[d]	93
Souza, 1993 BASF DocID C022615	50	Oral (10)	48	32	53	-[d]	-[d]	93
Laurent et al., 1983 BASF DocID C022987	200	Percutaneous (6)	24	0.2	0.1	<0.1	1.2	92[e]

[a] Includes cage washes
[b] Includes gastro-intestinal tract and skin
[c] Repeat dose: 14 daily doses followed by single dose of (14C)-iprodione
[d] Not measured in this study
[e] Includes unabsorbed dose

Absorption

Radioactive material was rapidly and highly absorbed following single oral doses of (^{14}C)-iprodione. In male rats, following a single oral dose at 50 mg/kg the highest mean concentration of radioactivity in the blood was found at 4 hours after administration (28.2 ± 6.8 µg equiv./g). For females similarly dosed, the highest mean concentration of radioactivity in the blood was found at 2 hours after administration (24.1 ± 3.5 µg equiv./g). In male rats, following single oral dose at 900 mg/kg, the highest mean concentration of radioactivity in the blood was found at 6 hours after administration (81.7 ± 11.8 µg equiv./g). In females at this dose level (900 mg/kg), the highest mean concentration of radioactivity in the blood was found at 6 hours after administration (71.6 ± 23.8 µg equiv./g). A conservative estimate of the degree of absorption can be taken from the percentage of the administered radioactivity eliminated via the urine. At the low dose level of 50 mg/kg these values ranged from 53% (females) to 67% (males) (BASF DocID C021582). These values are almost certainly under-estimates as the percutaneous administration study demonstrated the presence of radioactivity in the intestines and faeces 24 hours post-dose, which would be indicative of the presence of a biliary route of elimination (BASF DocID C022987).

Excretion

Rats dosed with radiolabelled iprodione at the rate of 100 mg/kg yielded a quantitative recovery of ^{14}C (higher than 99% for both sexes). There were indications that the elimination was faster in males than in females but for both sexes at 96 hours following dosing the same total radioactivity (approximately 98% of the dose) had been eliminated. The distribution of radioactivity by the routes of elimination were the same for both sexes, (62% in urine, 36% in faeces and less than 0.005% in exhaled air) (BASF DocID C022928).

For male rats which received (^{14}C)-iprodione (50 mg/kg) the mean blood concentration of radioactivity from 4 hours to 168 hours after administration declined to 0.16 ± 0.19 µg equiv./g. The mean elimination half-life was 8.9 ± 1.5 hours. For female rats which received (^{14}C)-iprodione (50 mg/kg) the mean blood concentration of radioactivity from 4 hours to 168 hours after administration declined to 0.035 ± 0.033 µg equiv./g. The mean elimination half-life was 6.9 ± 1.7 hours (BASF DocID C021582).

The mean blood concentration of radioactivity for the high oral dose group (900 mg/kg) males declined to 3.2 ± 5.84 µg equiv./g from 6 hours to 96 hours after administration. No radioactivity was detected in blood sampled at 120 to 168 hours. The mean elimination half-life was 19.8 ± 3.8 hours. The mean blood concentration of radioactivity for the high oral dose group females declined to 0.48 ± 1.08 µg equiv./g from 6 hours to 96 hours after administration. No radioactivity was detected in blood sampled at 120 to 168 hours. The mean elimination half-life was 12.5 ± 3.0 hours (BASF DocID C021582).

After a single oral administration of (^{14}C)-iprodione (50 mg/kg) the excretion of radioactive material via the urine (as a percentage of the radiochemical dose) was 67 % in males and 53 % in females. Excretion via the faeces was 25 % (males) and 39 % (females). After a single high dose (900 mg/kg), excretion via the urine was significantly lower in the males (43 %) but not in the females (46 %). The highest mean excretion of radioactivity in urine in all the mass balance groups was observed in the repeated low dose study: 75 % in males and 65 % in females. These values were significantly different from those in males and females in the high dose group. During the first 24 hours after administration of a single low dose, single high dose or repeated low doses, 53 %-73 % of the dose was excreted in urine and faeces of males and females. In the final sampling period (144-168 hours), the mean percentage of dose remaining in urine and faeces from any dose group was 0.3 % or less (BASF DocID C021582).

In the supplementary study after a single oral administration at 50 mg/kg the urinary elimination and faecal excretion were both major routes of excretion, the latter being quantitatively more important. Urinary elimination (including cage washes) accounted for 41.74 ± 8.55 % (males) and 37.43 ± 13.11% (females). Faecal excretion, primarily within 48 hours accounted for 56.06 ± 8.77 % and 50.36 ± 11.94 % in males and females respectively. The observed reduction in elimination via the urine is likely to be due to a reduced absorption, compared to the original study, caused by a difference in the particle size of the dose suspensions used (the methods of preparation being different) (BASF DocID C022615).

Distribution

The distribution of radioactive material in organs and tissues was extensive, but low, at 168 hours after single oral administration of 50 mg/kg or 900 mg/g of (^{14}C)-iprodione and after repeated administration of 50 mg/kg iprodione. The highest concentrations of radioactivity were in either the caecum or large intestine of males and females in all dose groups. No organs or tissues contained more than 0.05 % of the dose at this time except the skin (0.3%) of females in the repeated low dose study. As such a high proportion of radioactivity was excreted via the urine, some of the radioactivity found in the skin may have been due to contamination with urine (BASF DocID C021582).

These results are supported by those from the initial study where, apart from skin (approximately 0.5% of the dose) which was probably contaminated with excreta, liver from the females (0.1% of the dose) and the gastro-intestinal tract from the females (0.26% of the dose), no tissue analysed contained more than 0.09% of the dosed radioactivity (BASF DocID C022928).

Twenty-four hours after a single topical application, for both sexes, the amount of radioactivity that had penetrated through the skin for systemic circulation amounted to approximately 0.65% of the applied dose; up to 0.4% of this was eliminated in the urine and faeces in that period. Of the applied dose, 90.3% and 93.0% remained at the site of application and on the surgical plaster of males and females respectively (BASF DocID C022987).

A summary of the radioactive residue data available for the tissues after administration of (^{14}C)-iprodione in the rat is given in **Table 5.1.1-2.**

Table 5.1.1-2: Total radioactive residue concentration (µg equiv./g)

Tissue	Total radioactive Residue Concentration (µg equiv./g)							
	Single oral high dose		Single oral low dose		Repeated oral low dose		Percutaneous (a)	
	M	F	M	F	M	F	M	F
Liver	3.99	5	0.375	0.502	0.4	0.411	≤ 0.5	≤ 0.5
Stomach	0.402	1.3	0.01	0.154	0.043	0.08	0.7	< 0.5
Small Intestine	2.16	5.34	0.177	0.28	0.226	0.366	5.8(b)	6.1(b)
Large intestine	5.33	6.68	0.452	0.666	0.25	1.04	n.m.	n.m.
Caecum	6.72	9.9	0.429	0.694	0.418	1	n.m.	n.m.
Pancreas	1.57	1.54	0.057	0.145	0.184	0.161	n.m.	n.m.
Spleen	0.764	1.68	n.d.	0.028	n.d.	0.018	n.m.	n.m.
Kidney	2.44	2.11	0.207	0.209	0.301	0.199	< 0.5	4.8
Testis	0.656	n.a.	n.d.	n.a.	n.d.	n.a.	n.m.	n.m.
Uterus	n.a.	1.2	n.a.	0.136	n.a.	0.128	n.m.	n.m.
Lung	1.57	0.694	0.054	0.076	0.035	0.075	n.m.	n.m.
Heart	0.823	0.638	0.009	0.051	n.d.	0.049	n.m.	n.m.
Thymus	0.526	1.46	n.d.	0.04	n.d.	n.d.	n.m.	n.m.
Salivery gland	1.78	2.38	0.166	0.117	0.095	0.152	n.m.	n.m.
Muscle	0.157	0.319	0.012	0.01	n.d.	n.d.	n.m.	n.m.
Carcass	0.146	0.138	0.0251	0.012	n.d.	0.016	n.m.	n.m.
Skin	0.568	0.536	0.048	0.047	0.015	0.055		
Fat	2.44	2.54	0.163	0.226	0.296	0.877		
Bone	2.63	1.92	0.135	0.308	0.208	0.262	n.m.	n.m.
Eyes	0.105	0.05	0.017	0.015	0.004	n.d.	n.m.	n.m.
Adrenals	0.236	0.513	0.03	0.035	0.042	0.036	n.m.	n.m.
Thyroid	0.61	2.66	0.046	0.106	0.11	0.113	n.m.	n.m.
Lymph nodes	n.d.	1.75	n.d.	n.d.	n.d.	n.d.	n.m.	n.m.
Ovary	1.95	5.58	0.13	0.239	0.128	0.222	n.m.	n.m.
Blood	n.a.	1.86	n.a.	0.147	n.a.	0.139	n.m.	n.m.
Brain	1.62	0.754	0.103	0.109	0.135	0.072	0.65	1.25
Plasma	1.91	1.47	0.051	0.061	0.121	0.064	n.m.	n.m.

M Males
F Females
n.a. Not applicable
n.d. Not detected
n.m. Not measured
(a) Tissues taken 24-h after application as opposed to 168 hours post-dose for the oral groups
(b) Value includes both small and large intestine plus contents.

Metabolism
The results of the metabolite investigations as described below showed that the metabolism of iprodione is characterised by the large number of metabolites formed.

1) The metabolism of (^{14}C)-iprodione after oral administration to male and female rats at the dose level of 100 mg/kg was investigated in samples of excreta and some tissues where levels permitted (BASF DocID C022928). The results from this study indicated:
- *In the faeces*, in addition to iprodione, which is by far the most abundant compound (10% of the dosed radioactivity), six metabolites were detected in quantities of 1% to 2.2% (RP36115, RP32490, RP36114, RP36113 and two unidentified metabolites) in terms of dosed radioactivity. In addition, approximately 20 other radioactive compounds were detected, none of which exceeded 1% of the dosed radioactivity (total = 6.2% of dosed radioactivity). These included RP36110, RP36111, RP36112, RP36116 and RP36118.
- *In the urine,* in addition to iprodione (5% to 6% of the dosed radioactivity) 3 metabolites were found to which structures could be ascribed, namely RP32490, RP36114 and RP36119. In addition to these metabolites only two others (non identified) were found at respectively 2% and 2.8% of the dosed radioactivity. In common with faeces, urine was shown to contain a further 20 or so radioactive compounds but again none of these exceeded 1% of the dosed radioactivity (total 6.3% to 10.5% of the dosed radioactivity). These included RP30228, RP6110, RP36111, RP36112, RP361116, RP36117, RP36118 and RP36120. Results of urine analysis following enzyme treatment indicated that RP36114, RP36119 and some of the unidentified metabolites were present, in part, in the urine as conjugates.
- Where it was possible to analyse *tissues* (liver, gastro-intestinal tract and carcasses) on the basis of radioactivity content, there were indications that iprodione and RP30228 were present but it was not possible to quantify these with confidence because of the low levels present. (BASF DocID C022928).

2) The metabolism of (^{14}C)-iprodione after oral administration to male and female rats at the dose levels of 50 mg/kg (single and repeated doses) and 900 mg/kg (single dose) was investigated in samples of urine and faeces (BASF DocID C021582). The results from this study indicated:
- *Urine samples* from the low, high and repeated dose groups contained relatively little parent compound and a large proportion of unidentified (possibly conjugated) polar metabolites. The major metabolites (>10 % of the radioactivity in the sample) were further identified to be compounds RP32490 (de-alkylated derivative of iprodione) and RP36114 (produced by cleavage of the hydantoin ring in males and RP32490 and parent compound in females.
- *Faeces,* in males and females of all dose groups, contained a large (but minor, in the low dose group) proportion of parent compound. Differences in the proportion of the metabolites occurred between males and females, and between dose groups. In males, no major metabolites (> 10 % of sample) occurred between 0 and 24 hours. Between 24 and 48 hours, material which corresponded to either RP36115 or RP36119 (not resolved by chromatography) was observed for sample from all dose groups except that there were higher proportions of RP36114, particularly in the 24 to 48 hour period (BASF DocID C021582).

3) The metabolism of (^{14}C)-iprodione after oral administration to male and female rats at the dose level of 50 mg/kg (single) was investigated in samples of urine and faeces (BASF Doc C022615). Thin layer chromatographic and high performance liquid chromatography analysis were used to identify the metabolites present and confirmation was obtained by liquid chromatographymass spectrometry (LC-MS). The results from this study indicated:
- *Urine*, metabolite profiling of untreated urine indicated a very polar region which accounted for approximately 70 % of the sample radioactivity. Following deconjugation which was greater when a non-specific enzyme preparation was used i.e. a mix of ß-glucuronidase and aryl sulphatase, the proportion of conjugates were considerably reduced. Two major regions of radioactivity were seen but these were only partially resolved. LC-MS confirmed the presence of RP36115, RP32490 and RP36112 and indicated the presence of RP36119 and RP30228 in urine.
- *Faeces*, contained a large proportion of parent compound and almost all the remaining radioactive material chromatographed with reference articles. The major faecal metabolites which were confirmed by LC-MS were: RP36115, RP36114, RP32490 and RP30228.
In conclusion, TLC and HPLC profiling against reference articles characterised ca 80% of the sample radioactivity (23.57 % of the dose) in 24 hour male and female urine. Approximately 90% of the sample radioactivity (48.74 % of the dose) in the male and female faeces was characterised. Thus 72.31 % of the dose was identified, this represented almost 90 % of the total radioactivity in the samples profiled.

4) The metabolism of (^{14}C)-iprodione after percutaneous administration to male and female rats at the dose level of 50 mg/kg was investigated in samples of urine and faeces (BASF DocID C022987). The results from this study indicated:
- *Urine*: in addition to iprodione, three metabolites were detected and identified as RP32190, RP36114 and RP30228.
- *Faeces*: in addition to iprodione (detected in the faeces from the males only), two metabolites were observed: RP32490 and RP36114.
- *Tissues*, in the tissues where metabolite investigations were attempted only the extracts of the gastro-intestinal tract provided for confident interpretation. In this tissue and for both sexes, iprodione as well as RP32490 and RP36114 were detected.

A summary of the data obtained after oral gavage administration is given in **Table 5.1.1-3**. The data from the percutaneous administration was not included as identification was difficult due the very low levels of metabolites caused by a very low absorption of parent compound.

Table 5.1.1-3: Metabolite content of excreta

Compound	% Dose (mean data from male and female rats)					
	BASF DocID C022928		BASF DocID C021582		BASF DocID B003653	
	Urine	Faeces	Urine	Faeces[a]	Urine	Faeces
iprodione	6	10	3	13	n.i.	33.5
RP32490	20	2	10	2	15.2[b]	6.1
RP36112	≤ 1	≤ 1	1	1	15.2[b]	0.8
RP36114	11	2	8	3	n.i.	4.4
RP25040	≤ 1	n.i.	n.i.	<1	n.i.	n.i.
RP30228	≤ 1	n.i.	n.i.	<1	0.4	2.0
RP36111	≤ 1	≤ 1	n.i.	n.i.	n.i.	n.i.
RP36117	≤ 1	n.i.	n.i.	n.i.	n.i.	n.i.
RP36118	≤ 1	≤ 1	1	1	n.i.	0.8
RP36116	≤ 1	≤ 1	1	1	n.i.	n.i.
RP36113	n.i.	2	n.i.	n.i.	n.i.	n.i.
RP36115	n.i.	2	-[c]	-[c]	8	2.3
RP36110	≤ 1	≤ 1	n.i.	n.i.	n.i.	n.i.
RP36120	≤ 1	n.i.	n.i.	n.i.	n.i.	n.i.
RP36119	1	-[d]	-[c]	2	1.9	0.4
Unidentified Remainder	5[e] n.a.	3[e] n.a.	23[e] 7[f]	1[e] 8[f]	4.5[e] 0.2[f]	1.5[e] 0.1[f]
Total identified	47	23	25	23	26	50
Overall Total identified	70		48		76	
Comment	Allowing for 82% procedure efficiency = >95% of dose.		Virtually quantitative allowing for procedural losses: 0-48 hour period only		Urine results from TLC & faecal results from HPLC. LC-MS confirmation of metabolites. 0-48 hour period only.	

n.i. Not identified/detected
n.a. Not applicable
[a] Values corrected for extraction efficiency.
[b] RP32490 and RP36112 not resolved by TLC; LC-MS confirmed the presence of both metabolites
[c] Not resolved chromatographically
[d] Not proved to be present/absent
[e] Unidentified metabolites comprising low quantities of compounds dissimilar to reference compounds (probably conjugates)
[f] Unassigned radioactivity not accounted for as discrete peaks on chromatograms

Proposed metabolic pathway

A proposed general metabolic pathway for iprodione in the rat is presented in Figure 5.1.1-1 which is based upon the results from the studies discussed above.

The distribution of iprodione and/or related material as a result of absorption in organs and tissues was extensive but low at the end of the live phases of the oral studies at the dose levels used. The liver (up to 0.1% of the dose), gastro-intestinal tract (up to 0.26% of the dose) and skin (up to 0.5% of the dose) from rats used in the oral dosing studies were found to contain the greatest amount of dose related material. All other tissues and organs from the oral dosing studies were found to contain levels (as percent dose) ranging from < 0.001% to 0.05% at the end of the live phases.

The number and quantities of the metabolites (about twenty) detected in these studies showed that the proportion of the dose that was absorbed was extensively metabolised irrespective of dose level. For all three studies more unchanged iprodione was found in faeces than in urine which reflected the extensive metabolism undergone by absorbed iprodione.

The major metabolites found were:
RP32490 up to 22% of the dose
RP36114 up to 13% of the dose
RP36115 up to 10.3% of the dose
occurring in excreta and appeared to be end products of a chain of biotransformation mechanisms, several of these metabolites existing, in part, as a conjugate (glucuronide or sulphate).

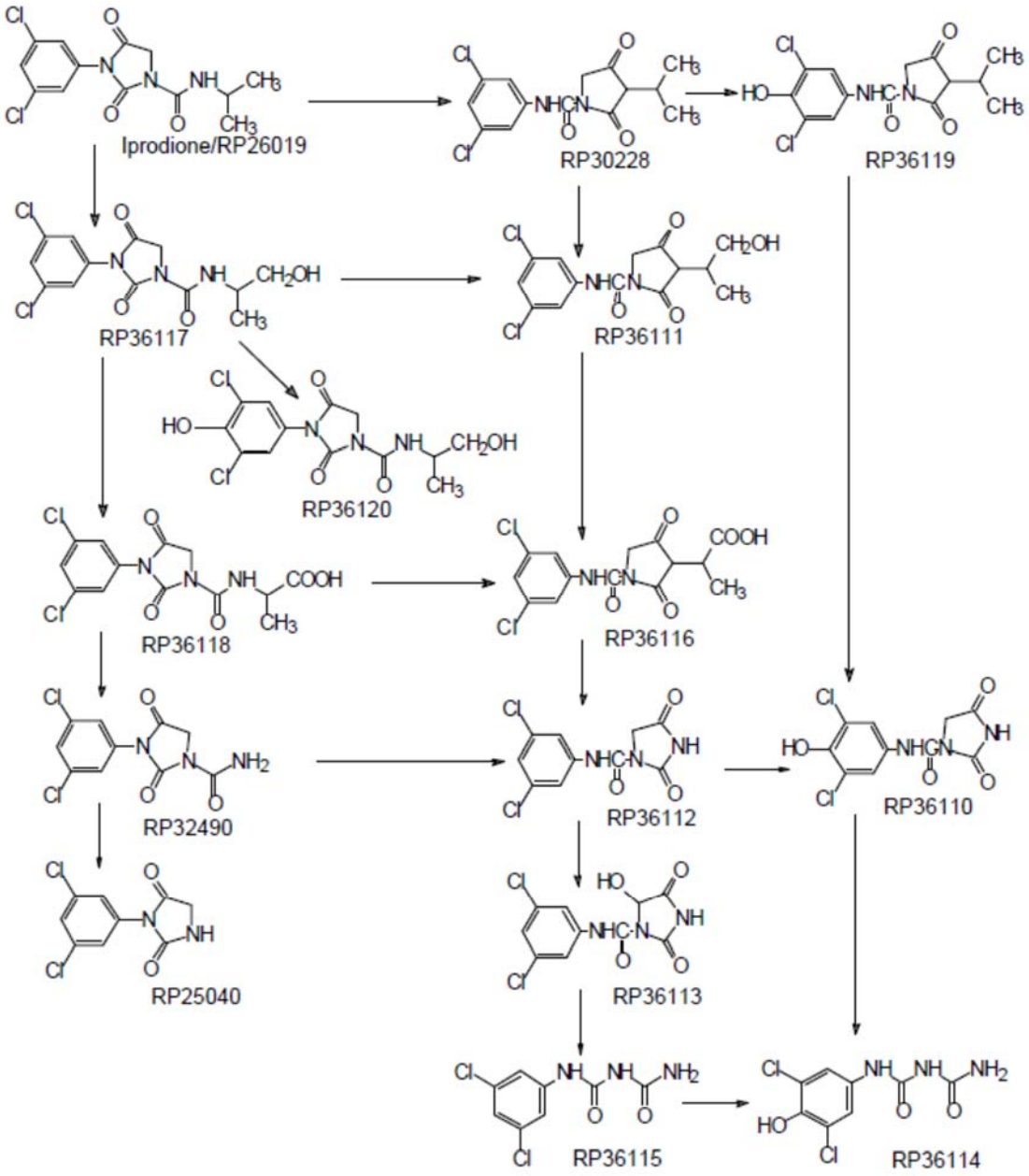
RP32490 (IUPAC): 3-(3,5-dichloro-phenyl)-2,4-dioxoimidazolidine-1-carboxylic acid amide
RP36114 (IUPAC): Allophanic acid (3,5-dichloro-4-hydroxy-phenyl)-amide
RP36115 (IUPAC): Allophanic acid (3,5-dichlorophenyl)-amide

The appearance of these metabolites, as well as iprodione and small quantities of others in faeces, as indicated in the percutaneous study suggests that enterohepatic circulation and elimination via the bile is a significant elimination route.

Evidence that the major metabolites are biotransformation end products is supported in that each metabolite which may considered to be an intermediate in such a sequence of biotransformation events is present in urine, or faeces, or both, in small amounts (up to approximately 2% of the dose). Such metabolites included RP30228, RP36111, RP36117, RP36118, RP36116, RP36112, RP36113, RP36110, RP36120 and RP36119.

The proposed general metabolic pathway for iprodione in the rat involves biotransformations that result in hydroxylation of the aromatic ring, degradation of the isopropylcarbamoyl chain and rearrangement followed by cleavage of the hydantoin moiety. Structural isomers of iprodione resulting from molecular rearrangement as well as intermediates in the pathway were also detected.

Figure 5.1.1-1: Proposed metabolic pathway of iprodione in the rat



Submission of not yet peer-reviewed studies in this AIR3-Dossier: In accordance with the requirements of Commission Regulation SANCO/11802/2010 and regulation (EC) No 1107/2009 an *in vitro* comparative metabolism study was performed and summarized below.

Report:	CA 5.1.1/1 Sweeney K, Geoffroy S, 2014a 14C-BAS 610 F: Comparative in vitro metabolism studies with rat and human liver microsomes 2013/1286147
Guidelines:	2004/10/EC of 11 February 2004
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

A comparative *in-vitro* metabolism study was performed with iprodione (BAS 610 F, Reg. No. 101169). The aim of the study was to compare the *in vitro* metabolism in microsomes of animal species used in toxicological testing of this substance to the metabolism in human microsome samples and to determine whether metabolic profiles are similar and whether a unique human metabolite occurs.

To answer this question, ^{14}C-labeled test item (mixed with non-labeled test item) at two concentrations (10 and 100 µM) was incubated with liver microsomes from human and rat samples (mixed gender) in the presence of an NADPH-generating system in order to enable Cytochrome P-450-related metabolism.

After incubation the supernatant was analysed by HPLC with radiodetection and selected samples were further investigated using HPLC-MS. These data were used to assign a retention time and an m/z-value to each peak that contains more than 5% of the applied radioactivity in human samples. To the corresponding peaks in animal microsome samples also retention time and m/z-value were assigned. The metabolite patterns formed with the animal microsome samples were compared to the metabolite pattern formed with human microsome samples to investigate whether a unique metabolite occurs in human microsome samples (above 5% of the applied radioactivity).

Negative and positive controls were tested in parallel to prove the metabolic activity of the liver microsomes and the absence of non-metabolic degradation. The active substance iprodione was extensively metabolised in all test systems and with human microsomes six peaks appeared in the HPLC-chromatogram that contained more than 5% of the applied radioactivity (based on the average of triplicates). M/z-values were assigned to each of these peaks.

All of these metabolites appeared in incubations with animal microsomes as well. Based on the procedures for comparison at a 5% of the applied radioactivity level, a unique human metabolite was not detected during this study.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**

Description:	iprodione (BAS 610 F)		
Batch # / purity:	(Phenyl-U-^{14}C) iprodione:	967-2022	99.1%
	Unlabeled iprodione:	COD-001260	97.8%
Stability of test compound	Stable during testing		

2. **Test system:** Microsomes
Positive control: microsomes were incubated with testosterone instead of the active substance to prove the metabolic activity of the microsomes

3. **Test animals:** Mammals
Species: Rat and human
Strain: Male and female Sprague Dawley rats (BD, United Kingdom)

B. STUDY DESIGN AND METHODS

The study was carried out at Quotient Bioresearch (Rushden) Ltd.

Test substance preparation
Stock solutions of ^{14}C-BAS 610 F were prepared in DMSO at nominal concentrations of 1 mM and 10 mM (i.e. 100x more concentrated than the target concentration in the incubation mixtures). To prepare 1 mM BAS 610 F application solution, 300 µL of ^{14}C-BAS 610 F was reduced to dryness and reconstituted in DMSO to prepare a 1 mM solution. For preparation of the 10 mM dose the radiolabeled material was diluted with non-radiolabeled material in order to prepare stock solutions with approximately the same activity/mL. To prepare the 10 mM BAS 610 F application solution, unlabelled BAS 610 F was dissolved in an aliquot of 1 mM ^{14}C-BAS 610 F to prepare a 10 mM solution.

Aliquots of each dosing solution were taken for liquid scintillation counting in order to confirm the radioactive concentration. Dose solutions were analysed by HPLC-MS in order to confirm the identity of BAS 610 F. The radiochemical purity was also assessed by HPLC-RAD on the day of the experiment.

Dosing solutions were stored at approximately -20°C whilst not in use and were assigned a nominal expiry date of 1 year.

Stock solutions of ^{14}C-testosterone were prepared in methanol at a nominal concentration of 15 mM (i.e. 200 x more concentrated than the target concentration in the incubation mixtures). To prepare the 15 mM ^{14}C-testosterone application solution, 500 µL of ^{14}C-testosterone was reduced to dryness under nitrogen and reconstituted in 15 mM solution of non-radiolabeled testosterone in methanol to prepare a 15 mM ^{14}C-testosterone solution (the amount or radiolabeled testosterone was considered negligible).

Microsomal preparations
Rat liver microsomes used in this study were purchased from BD Biosciences. For the tests individual batches of pooled male (n = 60 male rats) and pooled female (n = 23 female rats) microsomes were pooled in a ratio of 1:1, relative to the protein content to provide a mixed gender pool (n = 83 rats).

The human liver microsomes were already mixed gender pooled upon purchase. These microsomes comprised a mixture of microsomes prepared from individual donors (n = 150, male and female).

***In vitro* assays**
Preliminary assessment of non-specific binding
Prior to the *in vitro* metabolism assay, an assessment of the non-specific binding to the incubation vessels was performed. The test solutions (mix of unlabeled and ^{14}C-labeled BAS 610 F) were incubated at approximately 100 µM for high dose and at approximately 10 µM for low dose conditions with human liver microsomes.

Each sample comprised of microsomal protein, an NADPH generating system and potassium phosphate buffer (0.1 M, pH 7.4). The reactions were performed in tubes open to the atmosphere, at 37°C for up to 3 h in a shaking water bath. At 0 and 3 h aliquots of incubation mixture were taken for LSC and an assessment of non-specific binding to the incubation vessels made.

In vitro metabolism assays
The test solutions (mix of unlabeled and ^{14}C-labeled BAS 610 F) were incubated at approximately 100 µM for high dose and at approximately 10 µM for low dose conditions with rat or human liver microsomes.

Each sample comprised of microsomal protein, an NADPH generating system and potassium phosphate buffer (0.1 M, pH 7.4). The reactions were performed in tubes open to the atmosphere, at 37°C for up to 3 h in a shaking water bath and stopped by adding ethanol (methanol for positive controls). Additionally, for each species two negative controls and one positive control were performed.

The negative controls were a “heat denatured control” and a “t=0 control”. In addition a “buffer control” was performed. Under these conditions no metabolic degradation was expected. For the heat denatured control, the liver microsomes were inactivated by incubating in hot water (>95°C) for 30 min before pipetting them into the mixture. For the buffer control, the application solution was mixed only with buffer and NADPH. For the “t=0” control, the enzymatic reaction was stopped before adding the NADPH re-generating system by adding ethanol.

Incubation time-points were 0, 30, 60 and 180 min for human microsomes and 0 and 180 min for rat and control microsomes.

In the positive control, the different liver microsomes were incubated with ^{14}C-testosterone instead of the active substance to confirm the metabolic activity of the different liver microsomes. For the metabolic activity to be deemed acceptable, the following amounts of testosterone should be metabolised at least: rat 50%, human 50% (values based on previous experiments).

For each experimental setup, the treated samples in the two dose groups as well as all control samples were performed in triplicate.

II. RESULTS AND DISCUSSION

The triplicates of each control were comparable. In the three negative controls (buffer control, heat denatured control and t=0 control) the active substance iprodione was present along with other radioactive peaks indicating degradation of iprodione occurred without active microsomes. The transformation of iprodione under hydrolytic conditions is well known.

The positive control with testosterone showed that the metabolic activity of the microsomes was sufficiently high. The defined threshold values (greater than 50% metabolism) were reached in all cases.

After incubation for 3 h the reaction was stopped by addition of ethanol and the resulting supernatant was analysed before and after centrifugation. In the samples, the recovered radioactivity after centrifugation of the microsomal sample was compared to the radioactivity prior to centrifugation and was generally greater than 90% with the exception of one sample.

Six peaks contained more than 5% of the applied radioactivity in the human microsome samples. In Table 5.1.1-4 these six peaks in human microsome samples are compared with the peaks in animal microsome samples based on retention time and m/z-values (retention times and % of applied radioactivity are presented as mean values).

For the **first peak** above 5% of the applied radioactivity in the human microsome samples, the retention time of **8.8 min** could not be assigned to a m/z value in the positive-ion ESI-MS experiment. However, in a negative-ion ESI-MS experiment, the peak could be assigned to a m/z value of 242.97 with an isotope pattern characteristic for a double chlorinated compound. In human microsome samples this peak represents 10.06% of the applied radioactivity (10 µM), or 2.38% of the applied radioactivity (100 µM) following incubation for 180 min. This peak is also present in samples from the incubation with rat microsomes. In rat microsome samples this peak represents 8.47% of the applied radioactivity (10 µM), or 1.55% of the applied radioactivity (100 µM) following incubation for 180 min. In general this peak appears in the human microsome samples as well as in the animal microsome samples.

For the **second peak** above 5% of the applied radioactivity in the human microsome samples, the retention time of **11.7 min** was assigned to a m/z-value 304.06. In human microsome samples this peak represents 45.74% of the applied radioactivity (10 µM), or 8.95% of the applied radioactivity (100 µM) following incubation for 180 min. This peak is also present in samples from the incubation with rat microsomes. In all cases the amount of the applied radioactivity is above 5%. In rat microsome samples this peak represents 32.81% of the applied radioactivity (10 µM), or 6.86% of the applied radioactivity (100 µM) following incubation for 180 min. In general this peak appears in the human microsome samples as well as in the animal microsome samples.

For the **third peak** above 5% of the applied radioactivity in the human microsome samples, the retention time of **12.7 min** was assigned to m/z-values of both 364.05 and 346.04. It is believed that the latter peak results from an in-source loss of water from the molecular-ion peak m/z 364.05. In human microsome samples this peak represents 5.02% of the applied radioactivity (10 µM), or 0.96% of the applied radioactivity (100 µM) following incubation for 180 min. This peak is also present in samples from the incubation with rat microsomes. In rat microsome samples this peak represents 2.86% of the applied radioactivity (10 µM), or 1.02% of the applied radioactivity (100 µM) following incubation for 180 min. In general this peak appears in the human microsome samples as well as in the animal microsome samples.

For the **fourth peak** above 5% of the applied radioactivity in the human microsome samples, the retention time of **18.5 min** was assigned to m/z-values of both 348.05 and 432.01. It is believed that the m/z value of 432.01 represents an $(M+HCOO^-)Ca^{2+}$ adduct (with, overall, a single positive charge) of m/z 348.05. In human microsome samples this peak represents 15.27% of the applied radioactivity (10 µM), or 27.63% of the applied radioactivity (100 µM) following incubation for 180 min. This peak is also present in samples from the incubation with rat microsomes. In all cases the amount of the applied radioactivity is above 5%. In rat microsome samples this peak represents 17.32% of the applied radioactivity (10 µM), or 31.30% of the applied radioactivity (100 µM) following incubation for 180 min. In general this peak appears in the human microsome samples as well as in the animal microsome samples.

For the **fifth peak** above 5% of the applied radioactivity in the human microsome samples, the retention time of **19.8 min** was assigned to a m/z-value of 330.04. Based on the retention time and the m/z-value this peak is assigned to iprodione. The iprodione peak is present in all samples above 5% of the applied radioactivity: In human microsome samples this peak represents 15.14% of the applied radioactivity (10 µM), or 50.28% of the applied radioactivity (100 µM) following incubation for 180 min. In rat microsome samples this peak represents 20.45% of the applied radioactivity (10 µM), or 48.26% of the applied radioactivity (100 µM) following incubation for 180 min.

For the **sixth peak** above 5% of the applied radioactivity in the human microsome samples, the retention time of **23.4 min** was assigned as well as a m/z-value of 330.04. In human microsome samples this peak represents 2.97% of the applied radioactivity (10 µM), or 6.52% of the applied radioactivity (100 µM) following incubation for 180 min. In rat microsome samples this peak represents 5.54% of the applied radioactivity (10 µM), or 5.60% of the applied radioactivity (100 µM) following incubation for 180 min. In general this peak appears in the human microsome samples as well as in the animal microsome samples.

The underlying metabolites in the above mentioned peaks are thereby represented in the incubations with human microsomes as well as with animal microsomes.

Table 5.1.1-4: Comparison of major metabolites formed with human liver microsomes and rat liver microsomes following incubation for 180 min

Rt	m/z	Mean % applied radioactivity)			
		Human		Rat	
		10 µM	100 µM	10 µM	100 µM
8.8	242.97**	10.06	2.38	8.47	1.55
11.7	304.06	45.74	8.95	32.81	6.86
12.7	346.04 364.05	5.02	0.96	2.86	1.02
18.5	348.05 432.01	15.27	27.63	17.32	31.30
19.8	330.04	15.14	50.28	20.45	48.26
23.4	330.04	2.97	6.52	5.54	5.60

** detected in negative-ion ESI MS only

III. CONCLUSION

In summary, iprodione (BAS 610 F) is metabolised by liver microsomes of rats and humans under the investigated conditions.

Six peaks are present in human microsome samples at a level above 5% of the applied radioactivity (based on average of triplicates). For each of these peaks, a retention time and m/z-value was assigned. These metabolites do also appear after incubation with rat microsome samples which represent the species used for extensive toxicological testing.

Based on the comparisons at 5% of the applied radioactivity threshold, no unique human metabolite is detected within this study for iprodione.

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

One study investigating biokinetics and metabolism of radiolabelled iprodione was performed after topical (skin) application to rats (BASF DocID C022987). For convenience of the reviewer and similar to the iprodione monograph the results of this study are presented and discussed in the course of the summary of toxicokinetics after oral administration of iprodione to rats (see chapter 5.1.1 above).

CA 5.2 Acute Toxicity

Studies presented in the original Annex II Dossier (1995) or the update of the Annex II Dossier (1997): Iprodione has been tested in various species and via different routes of administration. All studies are scientifically valid; however, partially the studies have been conducted before the release of study guidelines and are without GLP according to the usual practice in those days. These studies have been evaluated by European authorities and France as Rapporteur Member State (European Commission Peer Review Program) and are, for the convenience of the reviewer, listed in Table 5.2-1.

Table 5.2-1: Summary of acute toxicity studies with Iprodione as available in the last evaluation

Study	Species/Sex	Test substance	Result	Reference
Acute toxicity Oral	Mouse, m/f	Iprodione	LD_{50}: 4000 mg/kg bw (m/f) 2000 mg/kg bw (m/f) 3000 mg/kg bw (m/f) 1870 mg/kg bw (m) 2670 mg/kg bw (f)	[redacted] 1974 1976 1976 1976 1976
	Rat, m/f	Iprodione	LD_{50}: > 3700 mg/kg bw (m/f) 2000 mg/kg bw (m) 1500 mg/kg bw (f) >2000 mg/kg bw (m/f)*	[redacted] 1976 1976 1976 1989
	Dog, m/f	Iprodione	LD_{50}: >2000 mg/kg bw (m/f)	[redacted] 1974
Acute Toxicity Dermal	Rat, m/f	Iprodione	LD_{50}: >2500 mg/kg bw (m/f)	[redacted] 1974
	Rabbit, m/f	Iprodione	LD_{50}: >1000 mg/kg bw (m/f) >2000 mg/kg bw (m/f)#	[redacted] 1974 1988
Acute Toxicity Inhalation, 4h	Rat, m/f	Iprodione	LC_{50}: > 3.29 mg/L (m+f) > 5.16 mg/L (m+f; whole body, 4-hours)$	[redacted] 1977 1993
Skin irritation	Rabbit	Iprodione	Not irritating	[redacted] 1974 1976
	Rabbit, New Zealand White	0.5 g Iprodione / animal	Not irritating	[redacted] 1991
Eye irritation	Rabbit	Iprodione	Not irritating to mildly transient irritating	[redacted] 1974 1976
	Rabbit, New Zealand White	0.1 mL Iprodione / animal	Not irritating	[redacted] 1991
Skin sensitization	Guinea pig	Iprodione	Not sensitizing	[redacted] . 1976
	Buehler test Guinea pig, Dunkin Hartley	Epidermal induction: 10% Challenge: 10% Rechallenge: 5%	Not sensitizing	[redacted] 1988

*: purity: 97.9%; #: purity: 95.8%; $: purity: 95.8%

Submission of not yet peer-reviewed studies in this AIR3-Dossier: A new sensitization study according to current criteria has been performed with iprodione for global registration as the available studies comprise a Buehler test, only. This study is submitted within this supplementary dossier. In accordance with the requirements of Commission Regulation SANCO/11802/2010 and regulation (EC) No 1107/2009 an in vitro NRU-Phototoxicity study in Balb/c 3T3 cells was not performed since the Ultraviolet/visible molar extinction/absorption coefficient of the active substance is less than 10 L × mol $^{-1}$ × cm $^{-1}$ (UV/VIS: Maximum 204.5 nm, ε = 44333 L mol^{-1} cm^{-1}. At 295 nm in acetonitrile ε less than 10 (see chapter 2.4)). New data available is tabulated in Table 5.2-2.

Table 5.2-2: Summary of newly available acute toxicity studies with iprodione

Type of study	Test substance	Result classification	Reference
Maximization test in guinea pigs	iprodione	Not sensitizing EU classification not required GHS classification not required	[redacted] 2010a BASF DocID 2010/1007141

Iprodione is of very low acute toxicity by the oral, dermal and inhalation route. It is not irritating to the skin and eyes and exhibits no effects of skin sensitization.

Based on the studies available at the time the EU agreed endpoints were:

Rat LD_{50} oral:	> 2000 mg/kg bw
Rat LD_{50} dermal:	> 2500 mg/kg bw
Rat LC_{50} inhalation:	> 5.16 mg/L (4-h, whole body)
Skin irritation:	Non-irritant
Eye irritation:	Non-irritant
Skin sensitization (Buehler and Maximization test)	Not sensitizing

With the newly available data, the relevant endpoints as proposed are:

Table 5.2-3: Relevant endpoints for acute toxicity of iprodione

Study type/species	Results	Classification	
		EU Dir. 67/548/EEC 2001/59 EC	Reg. EC 1272/2008 (CLP)
Acute oral toxicity, rat	LD_{50}: > 2000 mg/kg bw	-	-
Acute dermal toxicity, rat	LD_{50}: > 2500 mg/kg bw	-	-
Acute inhalation toxicity, rat	LC_{50}> 5.16 mg/L	-	-
Dermal irritation, rabbit	Not irritating to skin	-	-
Eye irritation, rabbit	Not irritating to eyes	-	-
Skin sensitisation Buehler and Maximization test	Not sensitizing	-	-
3T3 phototoxicity test	iprodione is not photoreactive	-	-

CA 5.2.1 Oral

The acute oral toxicity of iprodione has been evaluated during the Annex I listing of iprodione [see Table 5.2-1 for details].

CA 5.2.2 Dermal

The acute dermal toxicity of iprodione has been evaluated during the Annex I listing of iprodione [see Table 5.2-1 for details].

CA 5.2.3 Inhalation

The acute inhalation toxicity of iprodione has been evaluated during the Annex I listing of iprodione [see Table 5.2-1 for details].

CA 5.2.4 Skin irritation

The skin irritation potential of iprodione has been evaluated during the Annex I listing of iprodione [see Table 5.2-1 for details].

CA 5.2.5 Eye irritation

The eye irritation potential of iprodione has been evaluated during the Annex I listing of iprodione [see Table 5.2-1 for details].

CA 5.2.6 Skin sensitisation

The skin sensitising potential of iprodione has been evaluated during the Annex I listing of iprodione [see Table 5.2-1 for details]. The following study has been generated to satisfy a regulatory request in the global registration of iprodione.

Report:	CA 5.2.6/1 [redacted]., 2010a BAS 610 F (Iprodione) - Maximization test in guinea pigs 2010/1007141
Guidelines:	OECD 406, (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, EPA 870.2600, JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Bundesamt fuer Sicherheit im Gesundheitswesen, Wien, Austria)

Executive Summary

For the determination of potential sensitizing properties of BAS 610 F (batch: COD-000709, purity: 97.8%) a maximization test in Dunkin Hartley guinea pigs (Crl:HA) was conducted. Based on the results of a pre-test, the intradermal induction was performed with a 5% test item preparation in DMSO (5% in corn oil) into the neck region of the animals. The epicutaneous induction (7 days after intradermal induction) and the challenge exposure (14 days after epicutaneous induction) were performed with a 50 % test item preparation (1 g) in highly deionized water (w/w). The study was performed in 10 control and 20 test group animals. Readings were performed 24 hours after the intradermal injection and 24 hours after removal of the patch with regard to epicutaneous induction. Control group animals were treated with the same injection scheme as the test group animals but replacing the test item by the vehicle. Regarding epicutaneous induction, the control group animals were not treated with the vehicle (highly deionized water) since it was not expected to influence the result of the study. 14 days after the last induction, the challenge was carried out. 0.5 g of the 50% test substance preparation was applied for 24 hours to the intact skin of the flank under occlusive conditions. 24 and 48 hours after removal of the patch, skin readings were performed. A positive control with a known sensitizer was not included into the study. However, studies with Alpha-Hexylcinnamaldehyde (techn. 85%) are regularly performed as reliability check in the laboratory.
Discrete to moderate and confluent erythema were seen at the injections sites in all test group animals after intradermal induction. The epicutaneous induction with a 50 % test item preparation in highly deionized water (w/w) caused incrustation, partially open (caused by the intradermal induction) in addition to intense erythema and swelling in all test group animals. The challenge resulted in skin reactions in 0/10 control and 0/20 test group animals. The positive control Alpha-Hexylcinnamaldehyde (techn. 85%) was valid by showing a sensitization rate of 70% in the guinea pig strain.
The available data on skin sensitization 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). Therefore, no classification as skin sensitizer is warranted for BAS 610 F, based on the results obtained.

(DocID 2010/1007141)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 610 F

Description:	Solid, powder, white
Density:	not given
Lot/Batch #:	COD-000709
Purity:	97.8% (tolerance +/- 1.0%)
Stability of test compound:	Stable

2. Vehicle / Positive control:

Vehicles:
DMSO in corn oil: intradermal induction
Highly deionized water: epicutaneous induction and challenge
Positive control: Alpha-Hexylcinnamaldehyde

3. Test animals:

Species:	Guinea Pig
Strain:	Dunkin Hartley, Crl:HA
Sex:	female
Age:	5 - 8 weeks
Weight at dosing (mean):	286 - 347 g
Source:	Charles River Laboratories, Research Models and Services, Germany GmbH, Stolzenseeweg 32 - 36, 88353 Kisslegg
Acclimation period:	at least 5 days
Diet:	Ssniff Ms-H (Guinea Pig Maintenance Diet V2233-000), including ascorbic acid (2400 mg/kg), ad libitum
Water:	Tap water, ad libitum
Housing:	Groups of 6 or 11 animals were housed in plastic containers (48 cm x 115 cm x 36 cm), partly shaded

Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	Central air-conditioning
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: 12-Apr-2010 - 11-Jun-2010

2. Animal assignment and treatment:
The skin sensitizing potential of BAS 610 F was assessed using the Maximization Test based on the method of Magnusson and Kligman. For this, female guinea pigs were randomly allocated to groups. Ten animals were used as control group animals and 20 animals in the test group. Based on the results of a pre-test, animals were intradermally induced with 5% test substance preparations. Epidermal induction and challenge were conducted with 50% test substance preparations. The homogeneity and the stability of the test substance in the vehicle was determined indirectly by the concentration control analysis. The fur was clipped at least 2 hours before each test item application at the appropriate application sites. If necessary, the fur was additionally clipped at least 2 hours before evaluation of the skin reactions.

3. Clinical observation:
Mortality was checked once each day. No detailed clinical examination of the individual animals was performed but any obvious signs of systemic toxicity and/or local inflammation at the application sites were recorded.

4. Body weights:
Individual body weights were determined on day 0 on the last day of observation.

5. Pre-test:
1, 2.5 and 5% test substance concentrations were intradermally injected to three animals each. 6 intradermal injections in groups of two per animal were applied at the neck region: front row: 2 injections each of 0.1 mL Freund´s complete adjuvant without test item emulsified with 0.9 % aqueous NaCl-solution in a ratio of 1:1; middle row: 2 injections each of 0.1 mL of a test item preparation in vehicle at the selected concentration; back row: 2 injections each of 0.1 mL Freund's complete adjuvant / vehicle (1 : 1) with test item at the selected concentration. Skin reactions were assessed 24 and 48 hours after the beginning of the application. One week after the intradermal application, the same animals were used for the application in 1st epicutaneous pretest. For detecting a possible influence on irritating effects of previous intradermal treatment with Freund's complete adjuvant, three animals pretreated with Freund's complete adjuvant / vehicle (1 : 1) each, in the same manner as in the intradermal pretest 3 weeks prior to the application of the test item were additionally used within a 2nd epicutaneous pretest. For both pretests, 0.5 mL of the test item preparations (5, 10, 25 and 50%) were applied to the flank of each animal on fully loaded test patches (filter papers or Pur Zellin-Tupfer, Fa. Hartmann, 2355 Wiener Neudorf, Austria, ca. 2 cm x 2 cm). The patches were fixed with a strip of Fixomull® stretch" (self adhesive non woven fabric, hypoallergenic, made by Beiersdorf AG, 20245 Hamburg, Germany). Occlusion was obtained by covering with teflon foil which was fixed with Guinea Pig Jacket (Hugo Sachs Elektronik- Harvard Apparatus GmbH, Gruenestrasse 1, 79232 March-Hugstetten, Germany). The animals were exposed for 24 hours and skin readings were performed 24 and 48 h after removal of the patch.

6. Main study – intradermal induction:
Based on the results of the pretest, test group animals received intradermal injections of 5% test substance preparations analogously to the intradermal pretest (see above). Control group animals received the same injections but with the test substance preparation being replaced by the vehicle.

7. Main study – epicutaneous induction:
One week after intradermal induction, 1 g of the 50% test item preparations was applied to each test group animal under the same conditions as described in the epidermal pretest. The control animals were not treated with highly deionized water since the highly deionized water used as formulating agent was not expected to influence the result of the study.

8. Main study - challenge:
The challenge was carried out 14 days after the epicutaneous induction. 0.5 g of the 50% test item preparation was applied to the test and control group animal. The animals were exposed under occlusive conditions as described above for 24 hours and skin readings were performed 24 and 48 h after removal of the patch.

9. Evaluation of results
The number of animals with skin findings at 24 and/or 48 hours after the removal of the patch was taken into account for the determination of the sensitization rate. The evaluation "sensitizing" results if at least 30% of the test animals exhibit skin reactions.

10. Positive controls
A positive control (reliability check) with a known sensitizer was not included in this study. However, a separate study with the positive control Alpha-Hexylcinnamaldehyde (techn. 85%) is regularly performed in the laboratory.

II. RESULTS AND DISCUSSION

A. PRE-TEST

At intradermal induction, all concentrations were well-tolerated systemically. A test item concentration of 5% was the highest technically feasible concentration. At this concentration discrete erythema was observed. With regard to epicutaneous induction, no skin findings were observed in all animals treated with a 50, 25, 10 or 5% test item preparation 24 and 48 hours after the removal of the patch.

B. OBSERVATIONS

No abnormalities were observed during general observation.

C. BODY WEIGHTS

Body weight gain was not adversely affected during the course of the study.

D. SKIN REACTIONS AFTER INTRADERMAL INDUCTION

Injections of a 5% test item preparation in DMSO 5 % in corn oil caused discrete or patchy to moderate and confluent erythema in all test group animals (see Table 5.2.6-1).

Table 5.2.6-1: BAS 610 F - Skin reactions after intradermal induction

Position of injection: Neck region				
Form of application:				
Findings 24 hours after the beginning of application				
Animal #	**Application site**	**A) Freund's complete adjuvant / 0.9% aqueous NaCl Solution (1 : 1)**	**B) Test item 5 % in DMSO 5 % in corn oil (w/w)**	**C) Test item 5 % in DMSO 5 % in corn oil w/w/Freund's complete adjuvant (1 : 1)**
31	right	3	1	3
	left	3	0	3
32	right	3	2	3
	left	3	2	3
33	right	3	2	3
	left	3	2	3
34	right	3	2	3
	left	3	2	3
35	right	3	2	3
	left	3	2	3
36	right	3	2	3
	left	3	1	3
37	right	3	2	3
	left	3	2	3
38	right	3	2	3
	left	3	2	3
39	right	3	2	3
	left	3	2	3
40	right	3	2	3
	left	3	2	3
41	right	3	1	3
	left	3	1	3
42	right	3	2	3
	left	3	2	3
43	right	3	1	3
	left	3	2	3
44	right	3	1	3
	left	3	1	3
45	right	3	1	3
	left	3	1	3
46	right	3	1	3
	left	3	1	3
47	right	3	2	3
	left	3	2	3
48	right	3	2	3
	left	3	1	3
49	right	3	1	3
	left	3	1	3
50	right	3	1	3
	left	3	1	3

E. SKIN REACTIONS AFTER EPICUTANEOUS INDUCTION

The epicutaneous induction with a 50% test item preparation in highly deionized water (w/w) caused incrustation, partially open (caused by the intradermal induction) in addition to intense erythema and swelling in all test group animals (see Table 5.2.6-2). The control group animals, which were fixed with guinea pig jackets only, showed the same skin reactions as the test group animals.

Table 5.2.6-2: BAS 610 F - Skin reactions after epicutaneous induction

Animal #	Test item 50 % in highly deionized water
31	3
32	3
33	3
34	3
35	3
36	3
37	3
38	3
39	3
40	3
41	3
42	3
43	3
44	3
45	3
46	3
47	3
48	3
49	3
50	3

F. SKIN REACTIONS AFTER CHALLENGE

The challenge, which was conducted 14 days after the last induction, resulted in skin reactions in 0/10 control and 0/20 test group animals (see Table 5.2.6-3).

Table 5.2.6-3: BAS 610 F - Skin reactions after challenge

	Challenge			
Skin findings	**Control group**		**Test group**	
	24 h	**48 h**	**24 h**	**48 h**
Grade 0	10/10#	10/10	20/20	20/20
Grade 1	-	-	-	-
Grade 2	-	-	-	-
Grade 3	-	-	-	-
Swelling	-	-	-	-
Scaling	-	-	-	-
Severe scaling	-	-	-	-
Discoloration of the skin	-	-	-	-

x/y = number of findings / number of animals tested

G. POSITIVE CONTROL

The positive control Alpha-Hexylcinnamaldehyde showed a sensitization rate of 70% in the guinea pig strain. The results of the latest study conducted with the positive control are presented in Table 5.2.6-4.

Table 5.2.6-4: Skin reactions after challenge with the positive control

	Challenge			
Skin findings	**Alpha-Hexylcinnamaldehyde (techn. 85%) 10% in acetone**		**Vehicle Control: acetone**	
	24 h	**48 h**	**24 h**	**48 h**
Control group	10/5#	1/5	0/5	0/5
Test group	7/10	7/10	0/10	0/10

x/y = number of findings / number of animals tested (reading 24 h after application)

III. CONCLUSION

Based on the results of this study it is concluded that BAS 610 F does not have sensitizing properties in the guinea pig maximization test under the test conditions chosen. 0% of the animals were considered positive after challenge application.

CA 5.2.7 Phototoxicity

In accordance with the requirements of Commission Regulation SANCO/11802/2010 and regulation (EC) No 1107/2009 an in vitro NRU-Phototoxicity study in Balb/c 3T3 cells was not performed since the Ultraviolet/visible molar extinction/absorption coefficient of iprodione is less than 10 L × mol $^{-1}$ × cm $^{-1}$ (UV/VIS: Maximum 204.5 nm, ε = 44333 L mol^{-1} cm^{-1}. At 295 nm in acetonitrile ε less than 10 (see chapter 2.4)).

CA 5.3 Short-Term Toxicity

Studies presented in the original Annex II Dossier (1995) or the update of the Annex II Dossier (1997): Short-term toxicity studies (21 days – 52 weeks) with oral and dermal administration are available from four different species (rats, mice, dogs, rabbits). These studies have been evaluated by European authorities and France as Rapporteur member state (European Commission Peer Review Program) and were considered to be acceptable.

Table 5.3-1: Summary of oral and dermal subacute acceptable studies

Study	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Main effect	Reference and year
28-Day, oral CD-1 Mouse 0 - 1900 - 6000 - 9500 - 15000 ppm	270 (1900 ppm)	830 (6000 ppm)	LOEL: Liver enlargement, hepatocellular swelling, crystalline deposits in the urinary bladder. Higher doses: Mortality, increased liver weight.	[redacted] et al., 1979 BASF DocID R014614
21-Day, dermal New Zealand White rabbits 0 - 100 – 500 - 1000 mg/kg bw/day	100	500	LOEL and higher doses: Slight desquamation of the exposure site. Statistical differences in clinical chemistry data. Enlarged cervical lymph node in one female.	[redacted], 1991 BASF DocID C022490

Table 5.3-2: Summary of oral subchronic acceptable studies

Study	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Main effect	Reference and year
13-weeks, oral CD-1 Mouse 0 - 1500 - 3000 - 6000 and 12000 ppm	-	260 (male) 327 (female) (1500 ppm)	LOEL: Fibrous and mult-inucleate cell reaction to crystalline deposits in various organs. Centrilobular hepato-cyte enlargement. Enlarge-ment and/or vacuolation of *zona glomerulosa* and *fasciculata* of adrenals in female. Higher doses: Abdomen dark and/or swollen, lethargy, piloerection. Increase in liver, adrenal and spleen weight. Scattered, finely vacuolated hepatocytes. Uterine atrophy and absence of corpora lutea. Vacuolation of adrenals.	[redacted]., 1990b BASF DocID C022886
3-month, oral Rat 300 - 1000 and 3000 ppm (non-GLP study); 0 - 1000 - 2000 - 3000 and 5000 ppm (GLP-study)	20.5 (male) 23.7(female) (300 ppm))	78 (male) 89 (female) (1000 ppm)	LOEL: Slight but significant increase in mean liver weight of the female group. Slight impairment of food uti-lization. Higher doses: Decrease in body weight gain, uterus and ovary weight. Increase of liver weight and hepatocyte enlargement. Atrophy of the prostate. Vacuolation in the adrenals. Atrophic changes in the uterus and reduced numbers of corpora lutea in the ovaries. Testes interstitial cell hyperplasia.	[redacted]., 1978 BASF DocID C022485 [redacted]., 1990a BASF DocID C022531 & Amendment by [redacted] 1993 BASF DocID C022532
90-days, oral Sprague-Dawley rats 0 - 250 - 500 - 800 - 3000 ppm	30.81 (male) 35.79 (female) (500 ppm)	48.87 (male) 59.28 (female) (800 ppm)	LOEL: Body weight decrease. Histopathological changes. High dose: Body weight decrease. Clinical chemistry changes in females. Histopathological changes. Adrenal and prostate weight changes in males.	[redacted], 1997 BASF DocID R014676

Table 5.3-2: Summary of oral subchronic acceptable studies

Study	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Main effect	Reference and year
13-weeks, oral Beagle dogs 0 - 800 - 2400 - 7200 ppm (0 - 18 to 22 - 60 to 76 - 133 to 174 (180 to 205 during the last 7 weeks of the study) mg/kg bw/day)	60 to 76 (2400 ppm)	133 to 205 (7200 ppm)	LOEL: Slight hepatomegaly and increase in alkaline phosphatase.	[redacted], 1973 BASF DocID C021584 [redacted], 1977 BASF DocID C022488
52-weeks Dog; 0 - 100 – 600 - 3600 ppm	12.4 (male) 13.1 (female) (300 ppm)	24.6 (male) 26.4 (female) (600 ppm)	LOEL/Higher doses: Slight and occasionally significant reduction in RBC, Hb and Ht. Transient increase in Heinz bodies lower prostate weight. Slight adrenals and kidney microscopic changes. Haematologic changes. Higher liver and adrenal weights. Lower prostate weight Microscopic changes in adrenals, liver, kidney and urinary bladder.	[redacted], 1984/1985 BASF DocID C021579
52-weeks Dog; 200 - 300 - 400 - 600 ppm				[redacted], 1991 BASF DocID C021573

Results from subchronic studies (3-month) in the rat have indicated that the target organs were the adrenals, liver and the sex organs. Furthermore, signs of general toxicity such as decreased food intake and associated lower weight gain have been observed in animals treated from 2000 ppm. The clear No Observed Effect Level (NOEL) for subchronic oral toxicity in the rat was found to be 500 ppm, for both males and females, i.e. 33 mg/kg bw/day (approximately 30.81 mg/kg/day and 35.79 mg/kg/day, respectively).
Results from a first 52-week study in dogs indicated that dose levels of 600 ppm (24.6 mg/kg bw/d in males and 26.4 mg/kg bw/d in females) produced slight but marked effects. In a further study with 200 to 600 ppm (BASF DocID C021573), 600 ppm produced a slight reduction in RBC, HB and Ht which entails a conservative no-effect level at 300 ppm (12.4 mg/kg bw/d in males and 13.1 mg/kg bw/d in females).

Submission of not yet peer-reviewed studies in this AIR3-Dossier: No new subacute or subchronic studies with the active ingredient have been performed.

Based on the available studies, the following endpoints were determined in the Annex I listing of iprodione and are considered still valid:

Target / critical effect:	Liver, adrenal, testes, ovary, prostate, kidney, haematology, seminal vesicles (weight changes, hyperplasia, atrophy)
Lowest relevant oral NOAEL/NOEL	90-d rat: 31 mg/kg bw/day
Lowest relevant dermal NOAEL/NOEL	21-d rabbit: 100 mg/kg bw/day
Lowest relevant inhalation NOAEL/NOEL	No data, no study required

For convenience of the reviewer brief summaries of the respective studies as extracted from the monograph are provided under the respective chapters.

CA 5.3.1 28-day study

Mouse – 28 days

Ganter et al., 1979 (BASF DocID R014614): iprodione (26019 RP) One month toxicity study in mice by dietary administration

Groups of 15 males and 15 females CD-1 mice were fed with diets containing 0 - 1900 - 6000 - 9500 and 15000 ppm iprodione technical (purity 93.5%) for 4 weeks with 5 mice per sex at the 6000 and 9500 ppm levels being retained on study without further treatment for a 3 week withdrawal period. The object of the study was to confirm the presence and the nature of crystals seen in certain tissues in 2 previous studies using similar dose levels. The examinations performed were confined to the daily examination of the animals condition and behaviour and the weekly measurement of body weights and food consumption. The liver was the only organ weighed, but a full macroscopic and microscopic examination was made on all animals.
Most animals from the 15000 ppm group and a third of the females from the 9500 ppm group died during the study. Reduced food consumption and body weight gain were observed in surviving animals from these two groups particularly form the 15000 ppm group. The gross pathological examination showed increased liver weights at 6000 ppm and above and the presence of pale and/or mottled livers in some animals at 6000 ppm and above. White punctate spots were seen in the liver, heart and diaphragm in few survivors at 15000 ppm.
The histopathological examination revealed :
a) Foci of granulomatous formation associated with the presence of crystals
- in the liver of some animals at 15000 ppm,
- in the neck muscle, diaphragmatic muscle and myocardium of some animals at the 15000 ppm,
- in the subepithelial tissues of the urinary bladder in animals at 15000 - 9500 and 6000 ppm, but not at 1900 ppm.
These lesions were not present at 9500 ppm after the 3 week withdrawal period and so were considered reversible at this and lower dose levels.
b) Foci of hepatocytic hyperplasia seen at 15000 - 9500 and 6000 ppm, but not at 1900 ppm. These findings were associated with swollen vacuolated hepatocytes with marked variation in nuclear size and the presence of mitoses. After the 3 week withdrawal period slight swelling of the hepatocytes persisted at 6000 and 9500 ppm with slight nuclear variation in size.
c) Testicular changes at 15000 and 9500 ppm with a partial or total block of spermatogenesis at 15000 ppm in animals found dead during the course of the study and slight Leydig cell hyperplasia at 9500 ppm. These testicular abnormalities were not seen after the 3 week withdrawal period.
d) Atrophy of lymphoid centres in the spleen was observed in one male and one female surviving at the end of the treatment at both 15000 and 9500 ppm. This finding was also observed in some animals found dead during the course of the study.
Further pathological lesions seen were not dose related and were considered to be spontaneous in origin. A biochemical analysis of liver containing the crystals showed that these were probably crystals of RP 32490, a rat metabolite of iprodione.

Conclusion
The NOEL for this study is established at 1900 ppm (equivalent to 270 mg/kg bw/day) based on macroscopic and microscopic changes observed at 6000 ppm.

CA 5.3.2 Oral 90-day study

Mouse

Fryer et al., 1990b (BASF DocID C022886): iprodione: Sub-acute toxicity to mice by dietary administration for 13 weeks

iprodione technical (purity 95.7%) was administered to Charles River CD-1 mice (10/sex/dose) during 13 weeks at dietary concentrations of 0 - 1500 - 3000 - 6000 and 12000 ppm.
There were 4 deaths during the treatment period and 3 were from the high dose group and related to treatment. Treatment at 12000 ppm resulted in a loss of mean bodyweight during week 1 with subsequent recovery. Clinical symptoms in this group were mainly dark and swollen abdomen, lethargy, piloerection and hunched posture. Analysis of organ weights showed a dose-related increase in liver weights for all treated male and female mice compared to control mice. A significant increase in adrenal weights was also observed in males treated at 6000 ppm and above and in all treated female mice. A significant increase in spleen weights was observed in females at 3000 ppm and above and in males at 12000 ppm. When compared to control values, a decrease in organ weights was detected in the uterus at 3000 ppm and above, in the ovaries at 6000 ppm and above, and in kidneys of both males and females at 12000 ppm. Microscopic changes were observed at all treatment levels, the incidence and degree of change generally being greatest at 6000 and 12000 ppm. These changes consisted mainly of fibrous and multinucleate cell reaction to crystalline deposits in various organs. At 12000 ppm, this involves heart, liver, kidney, urinary bladder (often together with increased urothelial thickness), preputial gland, brain and skeletal muscle. In male, at 6000 ppm, this microscopic change was also seen in the urinary bladder together with increased urothelial thickness. Other microscopic changes included:
- centrilobular hepatocyte enlargement in male mice from all treated groups and in female treated at 3000 ppm and above. Scattered, finely vacuolated hepatocytes were present in male mice treated at 12000 ppm and female mice treated at 6000 ppm and above;
- uterine atrophy and absence of corporea lutea in females at 6000 and 12000 ppm;
- extramedullary hematopoiesis in the spleen of high dose group animals and in females at 6000 ppm;
- enlargement and/or vacuolation of the cells of *zona glomerulosa* and/or *zona fasciculata* was seen in the adrenals in female mice from all treated groups and in males from the high dose group. Females appeared to be more affected by these adrenal changes than male mice.

Conclusion
Because of organ weight and microscopic changes observed in the liver and adrenals at 1500 ppm, a NOEL could not be established. On the basis of the data showing clearly some non-specific reactions secondary to cristal deposits in several tissues with increased urothelial thickness in the urinary bladder, concentrations of 6000 ppm and above were considered not to be suitable for long term tumorigenicity investigations in mice.

Rat

Itabashi et al., 1978 (BASF DocID C022485): Three month dietary oral toxicity study of 26019 RP in rats

This early dietary study was conducted prior to GLP and was using dose levels of 300 - 1000 and 3000 ppm. Since then, more recent and full GLP subchronic and chronic studies, using higher dose levels, have been conducted in the rat.

Conclusion: The NOEL from this study was 300 ppm.

Fryer et al., 1990a (BASF DocID C022531): iprodione: Sub-acute toxicity to rats by dietary administration for 13 weeks & amendment
Gopinath and Ames, 1993 (BASF DocID C022532): iprodione RNP/322. Retrospective subacute toxicity to rats by dietary administration for 13 weeks

iprodione technical (purity 95.7%) was administered to Crl:CD (SD) rats (10/sex/dose) during 13 weeks at dietary concentrations of 0 - 1000 - 2000 - 3000 and 5000 ppm.
The 5000 ppm group had to be sacrificed during Week 8 due to a dramatic decrease in mean body weight gain (less than 20% of control) without indication of recovery and subsequent deterioration in clinical conditions. Therefore, the following clinical assessments are confined to the four other groups.
Treatment resulted in a decreased body weight gain and food consumption in both sexes of the 3000 ppm group and in males in the 2000 ppm group. Clinical signs were mainly confined to the 3000 ppm group and consisted of hunched posture, pilo-erection and/or cold extremities and emaciated appearance, generally from week 4. Treatment resulted in higher mean liver weight in all treatment groups. However, this was only significant in the female and after adjustment for body weight. A decrease in uterus weight at 3000 ppm and a decrease in ovary weight at 3000 and 2000 ppm were also observed. Microscopically, hepatocyte enlargement was observed in the liver of a few male rats treated at 3000 ppm. Vacuolation of the cells of the *zona fasciculata* and enlargement of the cells of the *zona glomerulosa* was observed in the adrenals of rats treated at 2000 ppm and above. Atrophic changes in the uterus and reduced number of *corpora lutea* in the ovaries were seen amongst female treated at 2000 ppm and over. An increased incidence of interstitial cell hyperplasia, atrophy of the prostate and reduced secretion in the seminal vesicles were observed in males at 3000 ppm. Atrophy of the prostate was also observed in one male at 2000 ppm. Although treatment at 1000 ppm resulted in a slight but significant increase in mean liver weight in female group, there were no macroscopic or microscopic changes, thus in the absence of any consistent changes, this was considered as a non-adverse effect level.

Conclusion
The results from subchronic rat studies have indicated that the target organs were the adrenals and the sex organs. Furthermore, signs of general toxicity such as decreased food intake and associated lower weight gain have been observed in animals treated from 2000 ppm. A slight impairment of food utilization was detected when animals were administered 1000 ppm. At this dietary concentration no macroscopic or microscopic changes were found except for a slight but statistically significant increase in the liver weight of the female group. The clear No Observed Effect Level (NOEL) for subchronic oral toxicity in the rat is 300 ppm corresponding to 21.9 mg/kg bodyweight/day) based on the initial 1978 study.

[redacted], 1997 (BASF DocID R014676): iprodione. 90-day toxicity study in the rat by dietary administration

iprodione technical (batch 9426801, purity 971 g/kg) was administered continuously *via* dietary administration to separate groups of Sprague-Dawley rats (10/sex/group) at concentrations of 250 - 500 - 800 and 3000 ppm for at least 90 days. A similarly constituted group of 10 males and 10 females received untreated diet and acted as a control. Clinical signs were recorded daily, body weight and food consumption were measured weekly. Ophthalmological examinations were performed on all animals during the acclimatization phase and on all animals of the control and high dose groups at week 12. The week before necropsy a blood sample was collected from the retro-orbital venous plexus of each animal for hematology and clinical chemistry determinations. Urine samples were collected overnight before necropsy from all animals. All animals were necropsied, selected organs weighed and a range of tissues were taken, fixed and examined microscopically.
There were no mortalities nor treatment-related clinical signs during the study. At the end of the study, a decrease of about 20% and 16% in body weight was observed at 3000 ppm for the males and the females, respectively, associated with a decrease in food consumption for the males only. A tendency towards body weight decrease was noticed in animals treated at 800 ppm. Administration of iprodione at 3000 ppm induced an increase in mean creatinine concentration in males and a decrease in glucose concentration in females. The significant histopathological findings were restricted to the animals treated at 800 and 3000 ppm. At 3000 ppm mean adrenal to body weight ratio was statistically significantly higher in males and pale adrenals were observed in a proportion of animals of both sexes; an increase in incidence and severity of vacuolation of both the *zona fasciculata* and the *zona glomerulosa* as well as a cellular hypertrophy of *zona fasciculata* were seen histologically. iprodione administered at 3000 ppm also induced minor histological testicular changes in 3 out of 10 males (slight to mid atrophy of the seminiferous tubules) and lower mean absolute and relative prostate weight, associated with a hyposecretion in two animals. An increased incidence and severity of corticocellular vacuolation in the adrenals were also noticed in the animals treated at 800 ppm.

Conclusion:
The No Observed Effect Level in this study was established at 500 ppm for both males and females (approximately 30.81 mg/kg bw/day and 35.79 mg/kg bw/day, respectively).

Dog

Coquet, 1973 (BASF DocID C021584): 3-month study of toxicity of 26019 RP orally in the dog

Groups of 4 Beagle dogs were subjected for 13 weeks to treatment of iprodione, administered in the diet at doses of 0 - 800 - 2400 and 7200 ppm (equivalent to 0 - 18 to 22 - 60 to 76 and 133 to 174 mg/kg bw/day for the first 6 weeks; for the last 7 weeks, the animals at 7200 ppm dose received the product directly in gelatin capsules equivalent to 180 -205 mg/kg/day). A change in the mode of treatment at Week 7 was necessary because of reduced food consumption due to palatability problem followed by a slight decrease in body weight gain. The use of capsules containing iprodione allowed a recovery in body weight gain in this group. Behaviour and consumption of feed were monitored daily. Bodyweight was determined each week. Ocular, hematological, biochemical and urinary examinations were carried out before, during and at the end of the treatment on all animals. Ultimately anatomo-pathological examinations were carried out on all animals (BASF DocID C022488).
From observations and examinations, apart from a slight increase in liver weight and an increase in plasma alkaline phosphatase in 3/4 animals treated at 7200 ppm, no evidence of toxicity was detected during the course and at the end of treatment.

Conclusion
The results from the 90-day study in the beagle dog indicated no evidence of toxicity when animals were administered 800 or 2400 ppm. At the next higher dietary level of 7200 ppm, slight hepatomegaly and increase in alkaline phosphatase were detected. The No-observed-effect-level (NOEL) for subchronic oral toxicity is 2400 pm corresponding to 67.8 mg/kg bw/day.

Broadmeadow et al., 1984 (BASF DocID C021579): iprodione: 52-week toxicity study in dietary administration to beagle dogs; Addendum 1 to LSR report no 84/RHO 022/179 (BASF DocID C021580); First supplement to LSR report no 84/RHO 022/179 (BASF DocID C021581).

Groups of six male and six female beagle dogs were fed iprodione (technical grade 96.5 %) added to their diet, at concentrations of 0 - 100 – 600 or 3600 ppm for 52 weeks.
No **clinical signs** were attributed to treatment. Food consumption, body weight gain, physical and ophthalmologic examinations and urinalyses revealed not treatment-related abnormalities. **Haematologic** changes were observed among dogs in the 3600 ppm group. These consisted of decreased (relative to control values), haemoglobin concentration, red blood cell count (RBC) and packed cell volume; and increased platelet count, partial thromboplastin time and an increased incidence of erythrocytes containing Heinz bodies. A slight and transient increase in red blood cells containing Heinz bodies was also observed in males treated at 600 ppm. No effects on hematological parameters were observed among dogs in the 100 ppm treatment groups. **Blood chemistry** values were similar among dogs in the control, 100 ppm and 600 ppm treatment groups. In the 3600 ppm group, alkaline phosphatase levels were significantly higher than control values at all sampling times for both males and females. Liver and adrenal **organ weights** in the 3600 ppm treated males and females were significantly higher than control values, indicating some degree of compound-related changes. Prostate weights among the male dogs in the 600 and 3600 ppm treatment group were significantly lower than in controls. The significance of this finding is unclear though its dose-related nature suggests that this prostate weight effect is probably treatment-related. No treatment-related findings were noted at necropsy among the dogs in the 100 ppm group. **Microscopic** changes at 3600 ppm was observed in the adrenals (increased depth of *zona fasciculata* and *zona glomerulosa* with large cells and "watery" cell cytoplasm), liver (increased frequency and size of agglomerates of pigmented macrophages and hepatic cord atrophy in centriacinar zones), kidney (increased frequency of lipofuscinosis in proximal convoluted tubules) and urinary bladder (granulomata containing crystals in the submucosa). Similar adrenal and kidney changes were also observed in dogs treated at 600 ppm. No treatment related-microscopic lesions were observed in dogs treated at 100 ppm.

Exposure to 3600 ppm in the diet for 52 weeks resulted in chronic toxicity as manifested most notably by histopathological changes in the livers, adrenals and urinary bladders. Administration of 600 ppm was associated with suggestive evidence for slight adverse effects. Therefore, the No-Observed-Effect-Level (NOEL) is 100 ppm (equivalent to 4.2 mg/kg bw/day).

Kangas 1991 (BASF DocID C021573): A 52 week dietary toxicity study of iprodione in the Beagle dog

A second dog study was conducted to define a more precise NOEL between 100 and 600 ppm. In this second dog study, the investigation was restricted primarily to the areas of concern to the US EPA which were blood parameters, prostate weights and possible ocular effects.
Male and female pure-bred beagle dogs (6/sex/dose) received iprodione technical, (purity 96.1 %) in the diet for 52 weeks. For male dogs, the average achieved intakes of iprodione at dietary levels of 200 - 300 - 400 and 600 ppm were 7.8 - 12.4 - 17.5 and 24.6 mg/kg bw/day, respectively. For female dogs the average achieved intakes of iprodione at dietary levels of 200 - 300 - 400 and 600 ppm were 9.1 - 13.1 - 18.4 and 26.4 mg/kg/day, respectively.
One male dog from the 600 ppm group **died** accidentally during week 8. This death was unrelated to treatment with iprodione. Scabbing and/or moderate to severe reddening of the skin was observed primarily in the inguinal, hindlimb and ventral abdominal regions in control and treated male dogs and in treated females. However no dose-response relationship for this **clinical sign** was found and the majority of the animals recovered from this affection by study termination. Treatment with iprodione was not associated with any marked changes in weekly mean body weights, overall body weight gains, or food consumption. When minimal changes were noted, they were always within the normal biological variation for dogs of this age. Statistically significant differences were occasionally observed for some treated animals in the group mean weekly feed efficiency when compared to controls. However, there was no significant trend indicative of a treatment-related effect. No ocular effects were noted at any time during the study which could be related to iprodione treatment. Slight and occasionally significant reductions in mean red blood cell count, hemoglobin, and hematocrit were observed in treated females and 600 ppm males when compared to control values. However for these groups, the **hematological** values recorded before treatment initiation were very similar to those observed during the study, and were always within normal biological ranges for beagle dogs of this age. Therefore these changes were not considered related to treatment. There was no incidence, at any time during the study, of Heinz bodies in erythrocytes from either control or treated animals. No evidence of a treatment-related effect was observed for adrenal, kidney and prostate **organ weights** at the end of the treatment period. Further male prostates showed no evidence of any treatment-related changes in the **gross and histopathological** results.

A No-Observed-Effect -Level (NOEL) of 300 ppm, corresponding to 12.4 mg/kg body weight/day for male and 13.1 mg/kg b.w./d for female was proposed.

Conclusion
Two studies were conducted. In the first one (BASF DocID C021579), a clear No Observed Effect Level (NOEL) was established at 100 ppm (4.2 mg/kg bw/day). In the second dog study (BASF DocID C021573), dose levels of 200 - 300 - 400 and 600 ppm produced no indications of a toxicological effect. Since the findings at 600 ppm in the first dog study were equivocal and were not observed in the second dog study, 600 ppm is probably a NOEL but this dietary concentration may be approaching a threshold level for toxicity.
Therefore based on the results from these two complementary studies, a conservative NOEL for the dog was established at 300 ppm (12.4 mg/kg body weight/day for male and 13.1 mg/kg b.w./d for female).

CA 5.3.3 Other routes

Rabbit: 21-days

Siglin, 1991 (BASF DocID C022490): 21-day dermal toxicity study in rabbits with iprodione technical

The potential toxic effects of iprodione technical (purity 96.2%) were evaluated in a 21-day dermal toxicity study in New Zealand White rabbits. The study consisted of a sham control group and three experimental groups with five animals per sex per group. The test article was ground to a fine powder and administered at levels of 100 - 500, and 1000 mg/kg bw/day. Gauze dressings were used to hold the test article in place. Each dressing was moistened with 10 mL of sterile water. Control animals received moistened gauze dressings only. The control and treated patched were maintained in place (dorsal area) for six hours per day for 7 days per week.
No mortality or clinical signs of toxicity were observed during the study. Slight desquamation of the exposure site was observed occasionally in a few animals in the 500 and 1000 mg/kg bw/day groups. By study termination (day 22), the exposure site of all treated animals were observed to be normal. No adverse effects were apparent based on body weight, body weight gain, food consumption and organ weights. Differences in hematology were sporadic of low magnitude, not dose-related.
Statistical differences on day 22 in clinical chemistry data consisted of a slight increase in cholesterol level in 500 and 1000 mg/kg bw/day females and increase globulin and total bilirubin in 1000 mg/kg bw/day females. Reddened thyroid/parathyroid in one 100 mg/kg bw/day female and two 500 mg/kg bw/day were observed and enlarged cervical lymph node in one 1000 mg/kg bw/day female. The only noteworthy microscopic effects were observed in the shin.

Conclusion
Based on the results described above, a NOEL of 100 mg/kg bw/day is considered.

CA 5.4 Genotoxicity Testing

Studies presented in the original Annex II Dossier (1995) or the update of the Annex II Dossier (1997): Iprodione has been tested both *in vitro* and *in vivo* in various and large number of mutagenic and genetic toxicity tests using bacterial, yeast, mammalian cells and whole animals to assess the genotoxic potential. Only the most reliable and full GLP studies are presented in tabular form in Table 5.4.1-1, Table 5.4.2-1 and Table 5.4.3-1and have been briefly summarized. These studies have been evaluated by European authorities and France as Rapporteur member state (European Commission Peer Review Program) and were considered to be acceptable.

Submission of not yet peer-reviewed studies in this AIR3-Dossier: No additional data on genotoxicity of Iprodione was generated.

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

CA 5.4.1 *In vitro* studies

Table 5.4.1-1: In vitro mutagenicity studies with Iprodione

Test System	Test Object	Conc.	Purity	Results	Reference
DNA damage	*B. subtilis*	20.6 to 1670 µg/disc	96.8%	Positive	Felkner, 1985
Rec-assay	*B. subtilis*	20 to 2000 µg/disc	99.4%	Negative	Shirasu et al., 1976
In vitro sister chromatid exchange	Chinese Hamster Ovary Cells	Activated: 5 to 400 µg/mL Non activated: 5 to 100 µg/mL	No data No data	Negative Negative	S. Sebastian, 1985b
DNA repair	*Escherichia coli*	Activated: 15.6 to 125 µg/plate Non activated: 15.6 to 125 µg/plate	95.1 %	Negative	Bouanchaud and Cartier, 1982b
	Escherichia coli	Activated: 25 to 200 µg/plate Non activated: 12.5 to 100 µg/plate	99.3%	Negative	Bouanchaud and Cartier, 1982a
Ames test (with and without activation)	*S.typhimurium* TA-98-100-1535-1537	12.5 to 100 µg/plate 1000 µg/spot	No data	Negative Negative	Benazet and Cartier, 1979
(without activation)		1 to 1000 µg/plate		Negative	
Ames test (with and without activation)	*S.typhimurium* TA-98-TA-100 TA-1535 TA-1537 TA-1538	25 to 200 µg/plate	99.3%	Negative	Bouanchaud and Cartier, 1982a
		25 to 200 µg/plate	95.1%	Negative	Bouanchaud and Cartier, 1982b
Ames test	*S.typhimurium*	Activated: 10 to 5000 µg/plate	96.2%	Negative	Lawlor and Valentine, 1990
		Non activated: 1 to 250 µg/plate		Negative	
Reverse mutation test	*S.typhimurium* TA98, TA100,TA1535 TA1537, TA 1538	100 to 1000 µg/plate	99.4%	Negative	Shirasu et al., 1976
CHO/HGPRT Mutation assay	Chinese Hamster Ovary Cells	Activated: 100 to 1500 µg/mL Non activated: 5 to 100 µg/mL	No data	Negative	Godek, 1985

Test System	Test Object	Conc.	Purity	Results	Reference
In vitro test in yeast (without activation)	*Saccharomyces cerevisiae* (D7)	250 µg/mL	No data	Negative	Benazet and Cartier, 1979
In vitro test in yeast (with and without activation)	*Saccharomyces cerevisiae* (D7)	125 - 250 - 500 µg/mL 62.5 - 125 - 250 µg/mL	99.3% 95.1%	Negative Negative	Bouanchaud and Cartier, 1982
In vitro chromosome aberration test	Chinese Hamster Ovary Cells	Activated: 40 - 150 - 400 µg/mL Non activated: 15 - 75 - 150 µg/mL	No data	Negative	S. Sebastian, 1985a
Induct test (with and without activation)	*Escherichia coli*	0.5 to 1000 µg/mL	99.3%	Negative	Bouanchaud and Cartier, 1982a
		0.05 to 1000 µg/mL	95.1%	Negative	Bouanchaud and Cartier, 1982b

CA 5.4.2 *In vivo* studies in somatic cells

Table 5.4.2-1: In vivo mutagenicity studies with Iprodione in somatic cells

Test System	Test Object	Conc.	Purity (%)	Results	Reference
Micronucleus Test	Male & Female CD-1 mice	750 - 1500 - 3000 mg/kg	96.1%	Negative	Proudlock and Elmore, 1994
Host metiated test	*S. typhimurium* (G46 his- strain)	250 - 500 mg/kg	No data	Negative	Shirasu et al., 1976

CA 5.4.3 *In vivo* studies in germ cells

Table 5.4.3-1: In vivo mutagenicity with Iprodione in germ cells

Test System	Test Object	Conc.	Purity (%)	Results	Reference
Dominant lethal test	Male mice	0-1500–6000 ppm	No data	Negative	Hastings et al., 1974

Overall conclusion

Iprodione has been tested both *in vitro* and *in vivo* in various and large number of mutagenic and genetic toxicity tests using bacterial, yeast, mammalian cells and whole animals to assess its potential to induce mutations and to damage chromosomes and DNA. With the exception of a single bacterial assay, all of these tests, including the DNA damage assay in mammalian cells, were clearly negative.
The single positive result was observed in an outdated assay designed to assess DNA damage using *Bacillus subtilis.* Because of major deficiencies in this study, the result found with iprodione in this assay is highly questionable i.e. inadequate negative and positive controls (the positive controls, with or without S9, were negative in this study), also the data, particularly, in the quantitative DNA damage assay showed considerable scatter, and there was problem of precipitation of the test material. Furthermore the assay itself is highly questionable because the parameter measured is inhibition of growth or cell death which is thought in this assay to be equivalent to DNA damage. But, distinguishing between cell death due to DNA damage or death due to other non-genetic mechanisms of toxicity, e.g. damage to cell membrane, is virtually impossible in the *B. subtilis* assay. The endpoint of cell death in the *B. subtilis* assay is too non-specific to reliably detect DNA damage. Thus, at present, this assay is considered to be outdated due to its high rate of false positives and the development of more sophisticated and reliable assays with mammalian cells to detect DNA damage/repair. Therefore, the results of this assay with iprodione are meaningless.

Thus, the weight of the evidence clearly supports that Iprodione does not pose a risk of inducing genetic mutations or DNA damage in humans:

Genotoxicity	No genotoxic potential

CA 5.5 Long-Term Toxicity and Carcinogenicity

Studies presented in the original Annex II Dossier (1995) or the update of the Annex II Dossier (1997): Long-term toxicity studies with oral administration are available from mice and rats. These studies have been evaluated by European authorities and France as Rapporteur Member State (European Commission Peer Review Program) and were considered to be acceptable. For the convenience of the reviewer, the studies are summarized in Table 5.5-1.

Table 5.5-1: Critical effects on long-term exposure/carcinogenicity as provided in the monograph

Study	NOEL (mg/kg bw/d)	LOEL (mg/kg bw/d)	Critical effects	Reference
99-weeks Mouse; 0 - 160 - 800 - 4000 ppm	23 (male) 27 (female) (160 ppm)	115 (male) 138 (female) (800 ppm)	LOEL: Minimal centrilobular hepatocyte enlargement in females. Vacuolation and hypertrophy of interstitial cells in testes. Hyperkeratosis of non-glandular stomach. High dose: Increased liver weight. Decreased uterus weight. Increased incidence of benign and malignant liver cell tumors (52% males, 42% females) and slight increase of luteomas in female ovaries (10%). Vacuolation and hypertrophy of interstitial cells in testes. Hyperkeratosis of non-glandular stomach. Haemosiderosis in spleen Amyloidosis and cortical scarring in female kidneys.	[redacted], 1993 BASF DocID C021564
104-weeks Rat; 0 - 150 – 300 - 1600 ppm	6.1 (male) 8.4 (female) (150 ppm)	12.2 (male) 17 (female) (300 ppm)	LOEL: Increased liver weight. Slight increase of small prostates. Centrilobular hepatocyte enlargement. Vacuolation of *zona fasciculata* (one female) and *zona reticularis* (some males) in adrenals. Interstitial cell hyperplasia in testes. High dose: The same kind of lesions plus: significantly increased testes weight. Increased incidence of interstitial cell tumors in testes (29/60) with cell hyperplasia and atrophy of seminiferous tubules. Seminal vesicles small and minimal content. Generalised vacuolization of *zona fasciculata* and *zona reticularis* in adrenals for males.	[redacted] 1992 BASF DocID R014586

Submission of not yet peer-reviewed studies in this AIR3-Dossier: No new long-term or cancerogenicity studies have been performed. However, study amendments, additional historical control data and further mechanistic studies have been performed after submission of the original dossier and are reported below, supporting the threshold based, non-genotoxic mode of action.

Brief study summaries, including study amendments are provided below. Moreover, mechanistic studies performed with iprodione not reported in the original dossier are summarized in MCA 5.8.2. Assessment of the potential endocrine related properties of iprodione which are most likely the causative effect of Leydig cell tumor formation have been discussed in MCA 5.8.3 of this dossier.

Four **long-term toxicity studies**, two in the **mouse**, two in the **rat**, were carried out: the first set of long-term studies in mice (BASF DocID C021565) and rats (BASF DocID C021549) showed no abnormal presence of tumors (both studies not mentioned in **Table 5.5-1)**. In short; **Mice** (Carworth CF-1 outbred albino mice) were fed diets containing 0 - 200 - 500 or 1250 ppm of iprodione for 18 months. iprodione did not affect body weight gains, food consumption, clinical observations of the mice in this study. Histological evaluation of all tissues showed that iprodione did not cause an increase in non-neoplastic pathology. The NOEL of iprodione in mice appeared to be greater than 1250 ppm (greater than 180 mg/kg bw/day) in that study. **Rats** (Charles River outbred CD albino rats) were fed diets containing 0 - 125 - 250 or 1000 ppm of iprodione for 24 months. High dose male rats and all the treated female rats showed a slight decrease in body weight gain when compared to corresponding control animals. Food consumption was significantly depressed in all treated rats, which probably accounted for the slight decrease in body weight gain. Chronic feeding of iprodione in rats did not affect the clinical chemistry, hematology or urinalysis values that were obtained throughout the study. The only apparent effect of iprodione on organ weights was a slight, but significant decrease in absolute and relative spleen weights in male rats sacrificed at 24 months. However, variation in organ weight did not show a group distribution and did not appear to be related to treatment. Iprodione did not increase the mortality rate nor did it cause an increase in neoplasms. Histological evaluation of all tissues showed that iprodione did not cause an increase in non-neoplastic lesions. The NOAEL of iprodione in rats appeared to be greater than 1000 ppm i.e. greater than 50 mg/kg bw/day in that study. Due to some deficiencies in these first studies, lack of a MTD according to US EPA criteria and lack of information on the purity, stability and homogeneity of the test material, new studies were performed.

Follow-up GLP studies were performed in mice (BASF DocID C021564 and amendment BASF DocID C021565) and rats ([see R014586 [redacted]. 1992 a] and amendments BASF DocID C022570 & C022572 & C022575). In mice, iprodione technical (purity 95.7%) was administered at dietary concentrations of 0 - 160 - 800 and 4000 ppm to groups of 50 CD-1 mice per sex, for 99 weeks. In addition to the main group, satellite groups of 15 mice per concentration per sex were used for interim sacrifice after 53 weeks. In rats, iprodione technical (purity 95.7 %) was administered at dietary concentrations of 0 - 150 – 300 and 1600 ppm to Sprague Dawley rats (60 animals /sex / dosage group) to evaluate the chronic toxicity and potential carcinogenicity in rats. In addition to the main group, satellite groups (10 rats/sex/dosage group) were used for interim sacrifice. Iprodione was found to induce liver tumors in mice and interstitial Leydig cell tumors in male rats. Luteoma in the ovaries of female mice were found with a higher incidence but within the historical control range (study amendment BASF DocID C021565). Each tumor type observed in the mouse and rat oncogenicity studies was detected in a single species, and the increased incidence of tumors was found only in animals treated at or above the MTD (mice 4000 ppm; rats 1600 ppm).

Based on the data provided in 1997, the list of EU agreed endpoints specified:

Target/critical effect:	Liver, Adrenals, Kidney, Testes, Ovary, Accessory Genital Organs, Extramedullary Hematopoïesis, Spleen Hemosiderosis (rat/mouse)
Lowest relevant NOAEL	2 year rat: 6.1 mg/kg bw/day
Carcinogenicity:	Rat: Interstitial Leydig cell tumors Mouse: Benign & malignant liver cell tumors in 2 sexes; Luteomas of ovary

Now available historical control data indicate that the observed slightly increased incidence of ovarian luteoma is incidental and therefore not related to treatment. It is therefore proposed to amend the list of endpoints as follows:

Target/critical effect:	Liver, Adrenals, Kidney, Testes, Ovary, Accessory Genital Organs, Extramedullary Hematopoïesis, Spleen Hemosiderosis (rat/mouse)
Lowest relevant NOAEL	2 year rat: 6.1 mg/kg bw/day
Carcinogenicity:	Rat: Interstitial Leydig cell tumors Mouse: Benign & malignant liver cell tumors in 2 sexes

Mouse

Chambers, et al. 1993 (BASF DocID C021564): Iprodione. Potential tumorigenic effects in prolonged dietary administration to mice (10 volumes)

This study was conducted because of some deficiencies in the previous oncogenicity study in mice (lack of a MTD according to US EPA criteria and lack of information on the purity, stability and homogeneity of the test material BASF DocID C021565).
Iprodione technical (purity 95.7%) was administered at dietary concentrations of 0 - 160 - 800 and 4000 ppm to groups of 50 CD-1 mice per sex, for 99 weeks. In addition to the main group, satellite groups of 15 mice per concentration per sex were used for interim sacrifice after 53 weeks.
There were no **clinical signs or mortality** related to treatment. At the highest dose level there was a significant decrease in body weight gain for both sexes compared to controls between Week 18 and Week 45. Before Week 18 and from Week 45 up to Week 99 group mean bodyweight gain for the treated groups was unaffected by the treatment with iprodione. There were no clear treatment related effects on **food consumption**. **Haematology** investigation at Weeks 52, 80 and 100 did not reveal any difference between treatment groups and control. **Clinical chemistry** investigation at Week 52 showed a significant increase of GOT and GPT values for both sexes receiving 4000 ppm. All the other groups were unaffected by treatment. Analysis of **organ weights** from animals killed for interim sacrifice revealed significantly increased liver weight (approximately 50% increase compared to control when adjusted to body weight) and slight increase in adrenal weights for both sexes receiving 4000 ppm. Decrease in uterine and ovarian weights was also observed at 4000 ppm although the difference from controls was not statistically significant. For animals killed after completion of 99 weeks of treatment, organ weights analysis revealed also significantly increased relative liver weight for males and females treated at 4000 ppm. There were also marginally increased thyroid weights for both sexes and significantly decreased uterus weights for females receiving 4000 ppm. A decrease in ovarian weights was also observed at 4000 ppm although not statistically significant.

Macroscopic observations after **interim sacrifice (52 weeks)** showed liver enlargement for both sexes treated with 4000 ppm and accentuated lobular markings in males treated at 800 ppm and males and females treated at 4000 ppm. Reduction of adipose tissues was also observed in females receiving 4000 ppm.
Examination of all animals **after 99 weeks** revealed the following changes:
- increased incidence of liver mass(es) for male mice treated with 800 ppm and for both sexes treated with 4000 ppm, with liver enlargement for both sexes of this last group,
- decreased incidence of thickening for uterus of female mice treated with 4000 ppm,
- slightly increased incidence of thickening and white appearance for forestomach amongst both sexes treated with 4000 ppm,
- slightly increased incidence of irregular cortical scarring of the kidneys and misshapen kidneys amongst female mice treated with 4000 ppm,
- increased incidence of masses, flacid and small testes for males treated with 4000 ppm.

Microscopic examination of interim sacrifice animals **after 52 weeks** showed no neoplastic findings considerable attributable to treatment. Non-neoplastic findings were only observed in mice treated with 4000 ppm and revealed the following changes related to treatment. In the liver, an increased incidence and degree of centrilobular hepatocyte enlargement for both sexes with increased incidence of centrilobular hepatocyte vacuolation in females of this group. In adrenals, an increased incidence of hypertrophy of the cells of the *zona fasciculata* was observed in females. In testes, generalised vacuolation and hypertrophy of the interstitial cells was observed in most of the males at 4000 ppm. In ovaries, luteinisation of the interstitial cells and absence of corpora lutea were observed in top dose females.
Microscopic examination of animals found dead, sacrificed in extremis, or killed at termination **after 99 weeks** revealed:
- an increased incidence of benign and malignant liver cell tumors amongst both sexes treated with 4000 ppm and
- a slight increase in the incidence of luteomas in female ovaries at 4000 ppm. The increased incidence of ovarian luteoma is within the historical control range (peer reviewed historical control data BASF DocID B003580, discussed below).

There was no treatment-related increase in neoplastic lesions with mice treated with 160 or 800 ppm.
Non neoplastic changes are summarised as follows:
- In liver: Single and multiple areas of enlarged eosinophilic hepatocytes, focal fat containing hepatocytes and centrilobular hepatocyte enlargement were present more frequently in both sexes treated at 4000 ppm with minimal centrilobular hepatocyte enlargement in female mice treated with 800 ppm. In male mice receiving 4000 ppm, pigmented macrophages were more frequently observed.
- In testes: an increased incidence of generalised vacuolation and hypertrophy of interstitial cells of the testes were noted in male mice treated with 4000 or 800 ppm.
- In ovaries: luteinisation of the interstitial cells of the ovary, absence of corporea lutea from the ovaries, arrest of follicular development, were more frequently noted in female mice treated with 4000 ppm.
- In stomach: an increased incidence of hyperkeratosis of the non-glandular stomach was noted in male mice treated with 4000 and 800 ppm.
- In spleen: haemosiderosis was more frequent in females treated with 4000 ppm.
- In kidneys: amyloidosis/amyloid deposits and cortical scarring were noted in female mice treated with 4000 ppm.

There were no treatment related changes in either sexes treated with 160 ppm.

Based on macroscopic and microscopic examinations, the NOEL for oncogenic effect is 800 ppm. Since none of the systemic effects observed at 800 and 4000 ppm were observed in the low dose group, the NOEL for toxicity is 160 ppm for males and females which is equivalent to a mean value 25 of mg/kg bw/day (23 and 27 mg/kg bw/day respectively for males and females (BASF DocID C021518; BASF DocID C021519).

BASF assessment of the mouse cancer study:
Review of ovarian lesions observed in the cancer study in CD-1 mice has been performed by a Pathology Working Group after submission of the original dossier showing that the luteoma incidence of all treatment groups lies within the historical control range and does not attain statistical significance ([see B003580 Hildebrandt P.K. 2001 a]). Therefore, slight increase in incidence of ovarian luteoma observed in the mouse cancer study at the highest dose tested is now assessed to be not treatment-related. A comprehensive position paper on the mechanism of toxicity and carcinogenicity in rodents was presented in the original dossier (BASF DocID R014641), concluding that tumors in the mouse were only observed at dose levels that exceeded the MTD.
Studies were conducted to elucidate the mode of action of the mouse liver tumorigenesis. In a first study (BASF DocID C022914), reported in the original dossier, the profile of liver changes observed following treatment with iprodione were very similar to the profile of changes observed after phenobarbital treatment, especially regarding the type of induced cytochrome P-450, the microscopic changes (centrilobular hypertrophy) and the sustained hepatocyte proliferation over a 14-day treatment period. In further mechanistic investigations (BASF DocID R014684), summarized in chapter MCA 5.8.2, a proliferative response in centrilobular hepatocytes was demonstrated in CD-1 mice following 13-day or 90-day dietary administration of iprodione to mice at the tumorigenic dose of 4000 ppm. No effects on cell proliferation were seen at dose levels of 60, 200 or 800 ppm iprodione. Based on the cancer study results and the mechanistic studies the overall conclusion is that liver tumors appeared secondary to toxicity and a clear threshold has been demonstrated for these effects.

Rat

[redacted], 1992 (BASF DocID R014586), amendment to the study report (BASF DocID C022570), additional historical control data (BASF DocIDs C022572; C022575): Potential tumorigenic and toxic effects in prolonged dietary administration to rats.

Because of some deficiencies in the first chronic toxicity oncogenicity study in rats (BASF DocID C021549; in particular the high dose level was below the MTD and there was no justification of doses and absence of homogeneity and stability data) a second study was conducted at the request of the U.S. E.P.A.
Iprodione technical (purity 95.7 %) was administered at dietary concentrations of 0 - 150 – 300 and 1600 ppm to Sprague Dawley rats (60 animals/sex/dosage group) to evaluate the chronic toxicity and potential carcinogenicity in rats. In addition to the main group, satellite groups (10 rats/sex/dosage group) were used for interim sacrifice.
The incidence and distribution of **mortality** and **clinical signs** was not related to treatment. At the highest dose level there was a significant decrease in weight gain for males compared to controls during the 22 first weeks after which the weight gain was comparable with control. Weight gain for females was also significantly reduced compared to controls but only for the first 12 weeks and again between weeks 22 to 82. Dietary administration resulted in a slight but consistent lower food consumption confined to male rats receiving 1600 ppm. Efficiency of food consumption was considered essentially comparable for the control and treated groups. **Haematology** and **clinical chemistry** investigations in weeks 26 - 52 - 78 and 104 did not reveal any differences from the controls that were considered to be related to treatment. Analysis of **organ weight** from animals killed for the interim sacrifice did not reveal any changes considered to be related to treatment, while animals killed after completion of 104 weeks of treatment revealed increased relative liver weight for males receiving 300 and 1600 ppm. Moreover, significantly increased testes weights were noted for males receiving 1600 ppm when compared with controls. A slight increase in relative thyroid and adrenal weights were also observed in males receiving 1600 ppm.

Macroscopic observations after **interim sacrifice (52 weeks)** did not reveal any changes related to treatment while examination of all animals **after 104 weeks** treatment (found dead, sacrificed *in extremis* or killed at termination) revealed
- masses in testes in 20/46 rats of the terminal kill treated with 1600 ppm compared with none amongst controls
- a slight increase in incidence of small prostates were noted for rats treated with 300 and 1600 ppm compared with none amongst controls.
- an increased incidence of seminal vesicles with minimal contents and small in size were noted with rats treated with 1600 ppm.
- in kidneys, a slight increased incidence in irregular cortical scarring was noted for male rats treated with 1600 ppm.
- an increased incidence of thickened uterus was also observed in females treated at 1600 ppm compared to control females.

Microscopic examination of interim sacrifice animals **after 52 weeks** showed that distribution of neoplastic findings, whose overall incidence was low, did not reveal any treatment related changes. Non-neoplastic findings were observed as follows:

- In liver, centrilobular hepatocyte enlargement was seen in male and female rats from the 1600 ppm and 300 ppm groups. In spleen, microscopic examination revealed an increased incidence of extramedullary haemopoiesis and haemosiderosis in female rats receiving 1600 ppm.
- In adrenals, generalised and/or focal enlargement of cells of *zona glomerulosa* were observed in numerous male and female rats treated with 1600 ppm. A high proportion of male and female rats treated with 1600 ppm revealed generalised rarefaction and fine vacuolation of *zona fasciculata*; only one female of the 300 ppm showed this latter change. A high proportion of male rats also showed a generalised fine vacuolation of *zona reticularis*. None of these changes in the adrenals were observed in control animals.

Microscopic examination of animals found dead, sacrificed in extremis, or killed at termination **after 104 weeks** revealed the following neoplastic lesions

- an increased incidence of interstitial cell tumors in testes amongst rats treated with 1600 ppm (29/60) when compared to controls (3/60).

Non-neoplastic changes are summarized as follows:

- in testes: an increased incidence of atrophy of seminiferous tubules was noted in rats treated with 1600 ppm. An increased incidence of interstitial cell hyperplasia was observed at 1600 ppm and 300 ppm. The increase of interstitial cell hyperplasia at 300 ppm is within the historical control range according to BASF DocID C022575.
- in epididymides: an increased incidence of reduced spermatozoa was noted in rats treated with 1600 ppm and 300 ppm. An increased incidence of absent spermatozoa was noted in rats treated with 1600 ppm. The increased incidence of reduced spermatozoa is within the historical control range whereas the increased incidence of absent spermatozoa is at the limit of the historical control range (BASF DocID C022575).
- an increased incidence of atrophy of the prostate was noted in rats treated with 1600 ppm,
- in seminal vesicles: an increased incidence of secretory colloid absent/empty or reduced secretion was noted in rats treated with 1600 ppm. Reduced secretion was also observed at 300 ppm.
- in spleen, the incidence of minimal haemosiderosis was increased amongst terminal female rats treated with 300 and 1600 ppm,
- in the adrenals: an increased incidence of either generalised or focal enlargement of cells of *zona glomerulosa* for males and females treated with 1600 ppm, often with generalized vacuolation of *zona fasciculata* and generalised vacuolation of *zona reticularis* for males treated with 1600 were noted. Generalised vacuolation of *zona reticularis* was also observed in male rats treated at 300 ppm.

Conclusion

Dietary administration of iprodione to rats at dose levels of 150, 300, and 1600 ppm during a 104-week period resulted in evidence of toxicity for male and female rats treated with 300 and 1600 ppm and an increased incidence of interstitial cell tumors in the testes of rats treated with 1600 ppm. Consequently, the No-Observed-Effect Level for oncogenic effect is considered to be 300 ppm. Since none of the effects observed at 300 and 1600 ppm were observed in the low dose group, the NOEL for toxicity was considered to be 150 ppm for males and females which is equivalent to a mean value of 7.25 mg/kg bw/day (6.1 and 8.4 mg/kg bw/day for males and females, respectively).

BASF assessment of the rat cancer study:
An amendment to the combined chronic- and cancerogenicity study in rats was published after submission of the original dossier, investigating early changes in the interstitium of the testes of male satellite animals killed after completion of 52 weeks of treatment ([see C022570 [redacted]. 1999 a]). This latter examination revealed a slightly increased incidence of minimal interstitial cell hyperplasia in the testes of males receiving 1600 ppm but no increase at lower dose levels.
Moreover a review of interstitial cell adenoma historical control data was performed in 1998 concluding that the incidence of interstitial cell adenomas in the iprodione treated low and intermediate dosage groups are within the historical control range ([see C022572 Gopinath C.,Offer J.M. 1998 a]) (see Table 5.5-2). Therefore, these results are considered to confirm the conclusion of the original report that the treatment-related increase in the interstitial cell adenoma was confined to the high dosage group.

Additional historical control data was provided after original dossier submission on incidences and/or severity of i) interstitial cell hyperplasia, ii) atrophy of seminiferous tubules iii) reduced spermatozoa and spermatozoa absent in epididymides as well as iv) adrenal enlargement and vacuolation ([see C022575 Ames S. 1996 a]) (see Table 5.5-2). These new data indicate that:

i) total number of Leydig cell hyperplasia was comparable between low- and mid-dose treatment groups (both 21.7%, vs. 11.7.% in controls) and only very marginally above the historical control range of 16 to 20%; the severity of cell hyperplasia was trace to minimal in the historical control, trace to moderate at low-dose (total of 2 animals displayed moderate hyperplasia), trace to minimal in mid-dose and trace to moderate at high-dose (total of 2 animals displayed moderate hyperplasia); overall there was no dose-related increase in severity of Leydig cell hyperplasia from 150 ppm to 1600 ppm.
ii) the statistically significant incidence of 40% atrophy of seminiferous tubules of high-dose males is within the historical control range of 10 to 48%.
iii) the incidence of reduced spermatozoa at mid- and high-dose in epididymides is within the historical control range (11.7% and 10% as compared to the historical control range of 0 to 32%). The incidence of absent spermatozoa at high-dose in epididymides is at the maximum of the historical control range (16.7% compared to the historical control range of 6 – 16%).
iv) the incidence at 150 ppm of focal enlargement of adrenal cortical cells of *zona glomerulosa* was not statistically significant with Fisher's test and only just outside of the historical control data and thus, considered to be of no toxicological significance. The incidence at 150 ppm of generalised fine vacuolation of the *zona reticularis* was not statistically significant on combined analysis of all animals in the group and is within the historical control data and thus, considered to be of no toxicological importance

Table 5.5-2: Selected testicular and adrenal effects in male rats of the combined chronic- and cancerogenicity study with iprodione

Dose [ppm]		0	150	300	1600
Animals in group		60	60	60	60
Leydig cell adenoma incidence[a]	**(%)**	3 (5)	7 (11.7)	7 (11.7)	**29* (48.3)**
Leydig cell hyperplasia					
- Descendent	(%)	2/29 (6.9)	6/25 (24)	1/28 (3.6)	**7/14** (50)**
- Terminal	(%)	5/31 (16.1)	7/35 (20)	**12/32** (37.5)**	**28/46** (61)**
- Total[b]	(%)	7/60 (11.7)	13/60 (21.7)	13/60 (21.7)	**35/60** (58)**
Mean severity grading[c]		(1.4)	(1.9)	(1.9)	(1.9)
Atrophy of seminiferous tubules[d]		10 (16.7)	14 (23.3)	14 (23.3)	**24** (40)**
Mean severity grading[e]		[2.6]	[2.6]	[2.8]	[3.3]
Epididymides					
Reduced spermatozoa[f]	(%)	2 (3.3)	3 (5)	**7* (11.7)**	**6* (10)**
Spermatozoa absent[g]	(%)	6 (10)	6 (10)	4 (6.7)	10 (16.7)
Adrenals (males)					
Focal enlargement of cells of *zona glomerulosa*[h]	(%)	1 (1.7)	6 (10)	9 (15)	**17** (28.3)**
Fine vacuolation of the *zona reticularis*[i]	(%)	7 (11.7)	14* (23.3)	**24** (40)**	**20** (33.3)**

[a] historical control range (0 - 15.4%)
[b] historical control range (16 - 20%)
[c] [ ] mean severity grading; histopathological findings were graded trace (Grade 1), minimal (Grade 2), moderate (Grade 3). The mean severity is the sum of the gradings divided by the incidence. Historical control range (1.0 – 1.6)
[d] historical control range (10 - 48%)
[e] [ ] mean severity grading; histopathological findings were graded trace (Grade 1), minimal (Grade 2), moderate (Grade 3), marked (4). The overall mean severity is the sum of the gradings divided by the incidence. Historical control range (2.6 – 3.4)
[f] historical control range (0 - 32%)
[g] historical control range (6 - 16%)
h historical control range (0 – 8%)
i historical control range (14 – 28%)
* $p < 0.05$; ** $p < 0.01$; (Pairwise Fisher's test)

A comprehensive position paper on the mechanism of toxicity and carcinogenicity in rodents was presented in the original dossier (BASF DocID R014641), concluding that the male benign interstitial cell tumors seen are only at the high dose in the 2-year rat study with iprodione and were due to a mode of action with a clear threshold. This conclusion was based on the following rationale:
i) The tumors were benign and only observed at a dose level at or above the MTD,
ii) Thresholds were demonstrated for both non-neoplastic and neoplastic lesions,
iii) The mechanistic toxicological research designed to elucidate the biochemical mode of action has shown that the primary biochemical lesion (inhibition of testosterone secretion) was a rapid and reversible phenomenon with a clear threshold and
iiii) The consensus of scientific experts was that benign Leydig cell tumors in the rat are not useful predictors of human disease. Thus, because the mechanism of action shows a clear threshold, and because the potential toxicological hazard has no direct relevance for human health, the dose response assessment for the benign interstitial cell effects in the rat testes should rely on threshold, non-linear, margin of exposure procedures.

A number of mechanistic studies to elucidate the mode of action and the threshold level of iprodione activity have been presented in the original dossier. Further studies have been performed after evaluation of the original dossier. Summaries of these studies are presented in chapter MCA 5.8.2. A comprehensive discussion and summary of all mechanistic studies is presented in chapter MCA 5.8.3 which also includes a review of relevant published literature. It is concluded that the mechanistic data for iprodione indicates a general inhibition of steroidogenesis which is the most likely causative effect for Leydig cell tumor formation. Moreover, a threshold for the endocrine related effects of iprodione has been identified, supporting the non-genotoxic, threshold based mode of action for tumor formation in the rat.

Literature review for the relevance of Leydig cell tumors to humans: Clegg et al (*Reproductive Toxicology 11(1), 107-121, 1997*) and Cook et al (*Critical Reviews in Toxicology, 29(2), 169-261, 1993*) consider that some categories of substances (specifically GnRH and dopamine agonists) causing Leydig cell tumors following disruption of the HPT axis are not relevant for humans. The potential relevance of other categories to humans, however, is not ruled out by these authors; however Cook et al consider the relative sensitivity of rats and humans to the proliferative stimulus initiated by elevated levels of circulating LH. Several aspects indicate that humans are likely to be much less sensitive to the induction of Leydig cell tumors than rats, including the following points:

The incidence of Leydig cell tumors in humans is much lower than seen in laboratory rat strains.

The incidence of Leydig cell tumors in humans is reported to be low. While this is true, the aspect of detection bias should be taken into account. Leydig cell tumors in rat carcinogenicity studies are detected as a result of detailed histopathology, whereas tumors in humans are likely only to be detected by palpation.

The spontaneous incidence of Leydig cell tumors in rat strains is reported to be approximately 5% in Wistar rats, 13.7% in Sprague-Dawley rats and approaching 100% in F344 rats (Nolte et al *Experimental & Toxicologic Pathology 63(7-8), 645-656, 2011*; Clegg et al (*Reproductive Toxicology 11(1), 107-121, 1997*) and Cook et al (*Critical Reviews in Toxicology, 29(2), 169-261, 1993*). The total incidence of testicular tumors in humans is 1% (Damjanov et al (*Annals of Clinical and Laboratory Science 9(2) 157-163, 1979)*; Cancer Research UK, 2008. *http://info.cancerresearchuk.org/cancerstats/types/*)); Leydig cell tumors account for 1-3% of all testicular neoplasms and occur in all age groups; approximately 20% are found between the ages of 5 and 10 with another peak between the ages of 30-35 (Skakkebaek et al (*Endocrinology of Male Reproduction (2003))*; Mati et al *International Urology & Nephrology 33, 103-105, 2002))* and appear to be more prevalent in white males between the ages of 20-60 (Al-Agha & Axiotis (*Arch Pathol Lab Med 131, 311-317, 2007)*; Odabas et al (*Eastern Journal of Medicine 3(2), 78-79, 1998))*. Cancer Research UK report that 95% of testicular tumors are germ-cell tumors, 4% are lymphomas and 1% are other histologies including Leydig cell tumors. In contrast to the rat, therefore, the human incidence of Leydig cell tumors is 0.01%, equivalent to an age-adjusted rate of 0.4 per million. A higher incidence of Leydig cell tumors is associated with certain medical conditions; an elevated incidence of 1% is reported in oligospermic men, in whom tumors were detected using ultrasonography and were not palpable, therefore it is possible that small Leydig cell tumors may escape detection and that the overall incidence in humans is somewhat higher than reported. Leonhartsberger et al (*BJU International 108 (1), 1603-1607, 2011*) also report that Leydig cell tumors may account for 14.7% of all testicular tumors when investigated using sensitive ultrasound technology. Clegg et al nevertheless conclude that the Leydig cell tumour incidence in humans is significantly lower than that in rats; Cook et al similarly make this conclusion, taking into account the potential for detection bias.

The absence of sex hormone binding globulin in the rat

Sex hormone binding globulin (SHBG) is a plasma protein which binds in excess of 95% of circulating testosterone in man, but which is absent in the rat. The extensive binding of testosterone in man has the effect of retarding its metabolism and clearance. Globulin-bound and bioavailable testosterone levels are kept in balance and, as a consequence of the difference in SHBG, circulating testosterone levels in man are relatively insensitive to short-term disturbances. In contrast, circulating testosterone levels in the rat are potentially more susceptible to perturbation. Clegg et al suggest that this marked species difference may be due to the relatively high mass of Leydig cells compared to blood volume in the rat. The rat testis is therefore considered to be potentially more susceptible to the xenobiotic-mediated disruption of circulating testosterone levels (Prentice & Meikle, *Human & Experimental Toxicology 14, 562-572, 1995*).

The number of LH receptors is lower in human testes

Human Leydig cells contain approximately 1500 LH receptors per cell, compared with approximately 20,000 per cell in the rat. Rat Leydig cells are therefore theoretically more sensitive to the effects of circulating LH; there is also experimental evidence supporting this hypothesis. Human and rat Leydig cells also respond differently to LH stimulation; hypertrophy is observed in both species, but hyperplasia is seen in the rat.

Rat Leydig cells also respond to GnRH

Unlike those in humans (and other species including other primate species and the mouse), Leydig cells in the rat contain GnRH (LHRH) receptors and may respond to GnRH directly.

Human Leydig cells are less sensitive to trophic stimuli

Testosterone levels decline with age in most rat strains and in humans, however the decline in rats is associated with declining LH levels whereas in man it is associated with increasing LH levels. In addition, the half-life of LH in the rat is 5-10 minutes, compared to 100 minutes in humans. The lower incidence of spontaneous Leydig cell tumors seen in humans is therefore associated with greater exposure to LH, indicating that human Leydig cells are inherently less sensitive to LH stimulation.

Conclusion: Iprodione appears to fit the profile of substances causing an increased incidence of Leydig cell hyperplasia and, following chronic administration, benign Leydig cell tumors in the rat. Findings are associated to increased stimulation of Leydig cells by LH, secondary to the reduced biosynthesis of testosterone. The markedly lower incidence of Leydig cell tumors in humans and the relative insensitivity of human Leydig cells to xenobiotic-mediated proliferation indicate that the increased incidence of Leydig cell tumors seen in the rat carcinogenicity study performed with iprodione are of limited relevance to the human risk assessment. A clear threshold is demonstrated for the effects of iprodione on the Leydig cell. Therefore, it is considered that the mode of action for Leydig cell carcinogenicity has been adequately elucidated in the rat. Data indicate a site of action, a temporal sequence of events consistent with the mode of action and a clear threshold for critical effects (hormonal changes, hyperplastic response and carcinogenesis). Published data clearly demonstrate species-specificity for Leydig cell carcinogenicity in the rat, which has a clear physiological basis. The data available for iprodione clearly indicate a species-specific effect which is consistent with general assumptions regarding Leydig cell carcinogenicity.

CA 5.6 Reproductive Toxicity

Studies presented in the original Annex II Dossier (1998): A two generation study in rat and developmental toxicity studies in rat and rabbit were part of the original Annex II dossier. The studies have been evaluated by European authorities and France as rapporteur member state (European Commission Peer Review Program) and were considered to be acceptable.

Submission of not yet peer-reviewed studies in this AIR3-Dossier: A new 2-generation study in the Wistar rat was conducted according to OECD TG 416 (2001) including enhanced parameters up to 1500 ppm (156 mg/kg bw/d) (BASF DocID 2013/1251918).

Table 5.6-1: Reproductive and Teratogenic studies performed with BAS 610 F

Type of study	NOEL (mg/kg bw/day)	LOEL (mg/kg bw/day)	Result with most sensitive species	Reference
Toxicity/Reproduction, 2-generation Sprague-Dawley rat 0 - 300 - 1000 - 3000 ppm	Parental toxicity, offspring and foetal toxicity: 18.5 (male) 22.8 (female) (300 ppm)	48.4 – 95.5 (males) 65.6 – 104.2 (females) 1000 ppm	LOEL and High dose: Depression of body weight gain in F0 and F1. Lower number of live pups/litter and lower pup weight. Poor health of pups.	Henwood, 1991 BASF DocID C021572
Toxicity/Reproduction, 2-generation Wistar rat 0 - 300 - 750 - 1500 ppm	Parental toxicity: 81 (750ppm) Developmental: ♂: 32 (300 ppm) ♀: 85 (750ppm) Reproduction: 156 (1500 ppm)	Parental toxicity: 156 (1500ppm) Developmental: ♂: 82 (750 ppm) ♀: 168 (1500ppm)	LOEL: Decreased food consumption and body weight/gain during gestation in parental. Reduced pre-weaning pup weight (♀); delay of puberty onset (♂).	[redacted] BASF DocID 2013/1251918
Teratogenicity Sprague-Dawley rat 40 - 90 - 200 mg/kg bw/day	90	200	LOEL: Slight lower fetal body weight and increased incidence of space between the body wall and organs. Higher doses (pre-test): Weight loss, pronounced clinical signs of pregnant rats. Litter resorptions. No teratogenic effect.	Tesh et al.,1986 BASF DocID C023030
Teratogenicity CD rats 20 - 120 - 250 mg/kg bw/day	20	120	LOEL: Macroscopic changes in the adrenals and a statistically significant reduction in body weight change. No teratogenic effects.	Repetto-Larsay 1997 BASF DocID C022658
Teratogenicity New Zealand White Rabbits 0 - 20 - 60 - 200 mg/kg bw/day	20	60	LOEL: Slight effect on maternal body weight. High dose: Maternal toxicity. Abortions. Post-implantation loss. No teratogenic effects.	Rodwell 1985 BASF DocID C032281

The reproductive toxicity of iprodione has been investigated in a number of studies in the rat, although the value of the older studies is somewhat limited by deficiencies in study design and/or reporting. In an older three generation study (BASF Doc ID C022999), no clear effects were observed on fertility or reproductive performance in the study, which was performed at dietary concentrations of up to 2000 ppm (120 mg/kg bw/d). No effects on reproductive performance at dietary concentration of iprodione as high as 3000 ppm were observed in a more recent 2-generation study reported in the original dossier (BASF DocID C021572); the NOEL for all effects in this study is 300 ppm (18.5 mg/kg bw/day males; 22.8 mg/kg bw/d females). A new study in the Wistar rat was conducted according to OECD TG 416 (2001) including enhanced parameters up to 1500 ppm (156 mg/kg bw/d) and summarized below (BASF DocID 2013/1251918). In short, the NOAEL for general systemic toxicity is 750 ppm (81 mg/kg bw/d) based on decreased food consumption and body weight/ body weight gain during gestation at the LOAEL of 1500 ppm (156 mg/kg bw/d). The NOAEL for fertility and reproductive performance is the highest dose tested (1500ppm; 156 mg/kg bw/d). The NOAEL for developmental toxicity in the female is 750 ppm (85 mg/kg bw/d) based on reduced pre-weaning pup body weights at 1500 ppm. In males the NOAEL for development is established at 300 ppm (32 mg/kg bw/d) due to delay in the onset of puberty at 750 ppm (82 mg/kg bw/d).

Teratogenicity of iprodione was assessed in rat and rabbit in a number of studies. Again, the value of the older studies is somewhat limited by deficiencies in study design and/or reporting. In these older studies, no embryotoxic or teratogenic effects at dose levels of 100 - 200 and 400 mg/kg bw/day were reported in the rat (BASF DocID C022944). In the rabbit iprodione was devoid of embryotoxic or teratogenic effect at dose level of 100 mg/kg bw/day. At dosages of 200 mg/kg bw/day and 400 mg/kg bw/day, some toxic effects were noted in the dams but no teratogenic effects were observed (BASF DocID C022989).
In more recent studies conducted, in both rats and rabbits iprodione was devoid of teratogenic activity:

- *In the rat*, no treatment-related maternal or fetal effects were observed at doses up to 200 mg/kg bw/day. iprodione showed no teratogenic property at any dose level in the rat, delayed fetal development was observed at 200 mg/kg bw/day. The NOEL for embryofetal toxicity is 90 mg/kg bw/day (BASF DocID C023030 and amendments BASF DocID R001054, BASF DocID C023031).
  A further developmental study was conducted on pregnant rats treated with iprodione from days 6 to 19 of gestation and at doses of 0, 20, 120 or 250 mg/kg bw/day (BASF DocID C022658). The results showed excessive mortality and/or maternal toxicity at 120 of 250 mg/kg bw/day, associated with slight reduction of the anogenital distance in male pups. A clear NOEL for maternal and foetal effects was demonstrated at 20 mg/kg bw/day.
- *In the rabbit,* no treatment-related embryotoxicity or teratogenicity was noted at doses of 20 or 60 mg/kg bw/day (BASF DocID C032281). Even though iprodione at 200 mg/kg bw/day was too maternally toxic for a complete teratologic evaluation, no malformations were observed in the fetuses obtained from this group. A loss in maternal body weight was noted in the 60 mg/kg bw/day group during the treatment period. Although this loss was not statistically different from the body weight gain observed in control animals, it could indicate slight maternal toxicity. Therefore, the clear no observable effect level for maternal toxicity and embryo/fetal toxicity is 20 mg/kg bw/day.

Based on the available data, the following endpoints were determined during the last Annex I listing of iprodione:

Target / critical effect - Reproduction:	Pup viability and weight at parentally toxic dose levels
Lowest relevant reproductive NOAEL / NOEL:	17 mg/kg bw/day
Target / critical effect - Developmental toxicity:	Rat: body weight; delayed development, ano-genital distance at maternal toxic dose levels. Rabbit: abortions, post implantation loss at maternal toxic dose levels.
Lowest relevant developmental NOAEL / NOEL:	20 mg/kg bw/day (rat and rabbit)

Based on the results of the new 2-generation study, the following list of endpoints is proposed:

Target / critical effect - Reproduction:	Pup viability and weight at parentally toxic dose levels
Lowest relevant reproductive NOAEL / NOEL:	17 mg/kg bw/day
Target / critical effect - Developmental toxicity:	Rat new 2-generation study: delayed sexual development, areola/nipples present at PND 20 Rat: body weight; delayed development, ano-genital distance at maternal toxic dose levels Rabbit: abortions, post implantation loss at maternal toxic dose levels.
Lowest relevant developmental NOAEL / NOEL:	20 mg/kg bw/day (rat and rabbit)

The available studies as provided in the monograph and the new 2-generation study in Wistar rat are summarized below.

CA 5.6.1 Generational studies

Henwood, 1991 (BASF DocID C021572): Two-generation reproduction study with iprodione technical in rats (Study presented in the original Annex II Dossier)

Due to deficiencies of the first generation study (BASF Doc ID C022999), including lack of histopathology on all parental animals of the high dose and control groups, justification of dose selection, demonstration of a maximum tolerated dose (MTD), appropriate dietary analyses and documentation of test material purity, a new study was initiated and reported in 1991.
Iprodione technical, (purity 96.2 %) was administered to Crl:CD Sprague-Dawley rats (F0 parents) via dietary admixture at levels of 0, 300, 1000 or 3000 ppm (28 rats/sex/group) for ten weeks prior to mating, throughout mating, gestation, lactation and weaning of F1a and F1b pups. F1a animals were selected to serve as parents of the F2 generation and received the same dietary concentrations as the F0 parents from weaning through at least ten weeks. At the time of mating of the F1 parents, the highest dietary concentration was lowered from 3000 ppm to 2000 ppm due to accumulating evidence (discussed below) that 3000 ppm exceeded the MTD. Dietary concentrations of 0, 300, 1000 and 2000 ppm were fed to F1 parents from initiation of mating through gestation, lactation and weaning of F2a and F2b pups. All parental animals and 10 weanlings/sex/group/generation were subjected to a complete necropsy. Selected tissues from high dose and control F0 and F1 parental animals were examined microscopically.
No adverse effects on reproductive performance were observed in this study. At 3000 ppm, significantly lower body weights and body weight gains were observed. During the period preceding the first mating of F0 parents, body weight gains were 30 to 40% below control. Body weight gains for F0 females during gestation of F1a and F1b pups were approximately 80 and 54% of control, respectively. Although body weight gains for F0 males during the production of F1b pups were comparable to control, their body weights remained about 80% of control. For the F1 parents, body weight gains were 20 to 40% below control during the time the animals were receiving 3000 ppm. Body weights for males and females during this same period were approximately 70 and 80% of control, respectively. The significant depression on body weight gain of parental animals and a decrease in pup survival in the first generation indicated that 3000 ppm exceeded the MTD. Therefore, the dietary concentration of 3000 ppm was lowered to 2000 ppm at the initiation of the F2a mating of F1 parents. After the dietary concentration was lowered to 2000 ppm, body weight gain for F1 males was comparable to control and their body weights were about 80% of control weights. For F1 females, weight gains during gestation of F2a and F2b litters were 86 and 79% of control, respectively. At 1000 ppm, body weight gains for F0 and F1 females were significantly lower than control during the premating periods. Food consumption was decreased for F0 and F1 parents in the high dose group throughout most of the study. At 1000 ppm, food consumption was also reported to be lower for several weeks for the F0 parents prior to the F1a mating and F1 females prior to the F2a mating. No treatment-related lesions were observed in high dose F0 and F1 animals at necropsy or upon histopathological examination. No treatment-related differences in mating indices or male or female fertility were observed. No effects on litter or pup parameters were noted at 300 or 1000 ppm.

However, at the highest dietary concentration, effects on pup weight, the number of live pups/litter and pup survival were noted. For the F1a litters, the number of live pups/litter at 3000 ppm was statistically lower on lactation day 0 compared to control. During lactation, 14 high dose pups were found dead, missing, or cannibalized compared to 3 control pups. In addition, 22 high dose pups from 7 litters were found dead, cannibalized, or missing after lactation day 21. Mean body weights for F1a pups were approximately 90% of control on lactation day 0 and 70% by lactation day 21. Clinical observations for F1a pups at 3000 ppm included small in size, slow moving, and generally unkempt appearance or in poor health (sparse or unkempt haircoat, dry brown material around eyes and nose). For F1b pups in the high dose group, the mean number of pups delivered (live and dead) and the mean number of pups/litter prior to culling on lactation day 4 were statistically lower than control. At parturition, 33 high dose pups were reported as stillborn compared to 14 control pups. In addition, 78 high dose pups (approximately 40% mortality) were found dead, missing, or cannibalized during the lactation period. Mean body weights for F1b pups were approximately 85% of control on lactation day 0 and 80% of control by lactation day 21. Clinical observations for F1b high dose pups consisted of hunching, tremors, and small in size. For the F2a high dose pups, significantly lower mean numbers of pups delivered (live and dead) and pups/litter on lactation day 0 were noted. For F2b pups in the high dose group, the mean number of pups delivered (live and dead) and the mean number of pups/litter prior to culling on lactation day 4 were statistically lower than control.
Mean body weights for high dose pups from the F2a and F2b litters were approximately 95% of control on lactation day 0 and 80% of control by lactation day 21. Pups from these litters were also reported as being small in size, hunched, and slow moving. Necropsy of ten weanlings/sex/litter/generation did not reveal any gross lesions attributable to treatment.

Conclusion
No effect on reproductive performance was observed at dietary concentrations of iprodione as high as 3000 ppm. Evidence of parental toxicity was observed at dietary levels of 1000 ppm and higher. Effects on pup viability and pup weight were noted at 2000 and 3000 ppm. The No Observed Effect Level for all effects in this study is 300 ppm.

(Study not yet peer-reviewed)

Report:	CA 5.6.1/1 [redacted] 2014a BAS 610 F (Iprodione) - Two-Generation reproduction study in Wistar rats - Administration via the diet 2013/1251918
Guidelines:	(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, EPA 870.3800, OECD 416
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 2-generation reproduction toxicity study, BAS 610 F (iprodione; Batch: COD-001260; Purity 97.8%) was administered in the diet to groups of 25 male and 25 female Crl:WI(Han) Wistar rats at nominal dose levels of 0, 300, 750 and 1500 ppm throughout 2 generations. The dietary concentrations of BAS 610 F were adjusted to 0, 150, 375, 750 ppm during lactation, thereby maintaining constant dose-levels during this period of increased food intake. The overall mean dose of BAS 610 F administered to the male and female Wistar rats during the entire study period was approx. 33 mg/kg body weight/day (mg/kg bw/d) in the 300 ppm group, approx. 83 mg/kg bw/d in the 750 ppm group and approx. 156 mg/kg bw/d in the 1500 ppm group. When enumerated for each sex the corresponding dose levels were approximately 32, 82 and 144 mg/kg bw/d for the males and 34, 85 and 168 mg/kg bw/d for the females. Due to particular findings in the $\mathbf{F_1}$ generation relating to anlagen of nipples or areolae in male offspring which were present beyond postnatal day 20, anogenital distance/index and age at sexual maturation the study protocol was amended to include additional examination of $\mathbf{F_2}$ offspring, which were then raised until sexual maturation. A subset of $\mathbf{F_2}$ offspring was further selected to be raised and mated with new control females to produce a $\mathbf{F_3}$ litter.

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 impaired in high dose $\mathbf{F_0}$ and $\mathbf{F_1}$ female animals during gestation.

Treatment with BAS 610 F up to the target concentration of 1500 ppm had no effect on the estrous cycle or female fertility. Regarding sperm analysis, no alteration was observed in males of the $\mathbf{F_0}$ generation, but males of the $\mathbf{F_1}$ generation had a slight but statistical significant increase in abnormal sperms in the cauda epididymidis compared to controls. On an individual animal basis a single high dose male was above the control and historical control range. All other animals were either within or at the border of the concurrent and/or historical control range. Neither effects on fertility nor histopathological changes were noted in the contralateral testes or epididymides. Therefore this finding is considered of no biological relevance. Overall, male and female fertility indices ranged between 92 and 100% without any relation to dose.

BAS 610 F 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 (96 to 100%). The observed numerical differences displayed no dose-response relationship and were thus not indicative of a relation to treatment. The statistical significant increased incidence of stillborn pups in mid-dose $\mathbf{F_2}$ females which displayed no dose relationship and is within the historical control range is due to a single dam with all stillborn pups. Therefore this finding is regarded incidental and not treatment related. Finally, ovarian follicle counts did not reveal any differences between control and high dose groups.

Survival of pups was not affected by treatment as viability and lactation indices in the range of 99 to 100% without dose relation in any generation show. Body weight development of high dose $\mathbf{F_1}$ and $\mathbf{F_2}$ pups was significantly impaired towards weaning or from mid-lactation on, respectively. The apparent reduction of anogenital distances/indices in high-dose $\mathbf{F_2}$ males was comparable in magnitude to high-dose $\mathbf{F_1}$ generation males thereby leading to the conclusion the changes are likely secondary to a reduction in pup body size rather than a compound specific effect on the anogential distance. Nipple / areola anlagen in $\mathbf{F_1}$ male pups (one mid-dose, four high-dose) and $\mathbf{F_2}$ male pups (five high-dose) were present at PND 20. However, follow-up of the affected single mid-dose $\mathbf{F_1}$ and five high-dose $\mathbf{F_2}$ pups, which were mated to control females giving rise to a $\mathbf{F_3}$ generation, displayed no effects different from control animals on clinical observation or different from group mean on onset of puberty or any other parameter assessed (macroscopic/ microscopic observations; mating performance; post-implantation loss) including observation of offspring from affected male parents (sex ratio; pup survival; presence of nipples / areolas at PND 20; sperm parameters). Moreover, mammary fatpads from affected $\mathbf{F_2}$ male animals were collected for further analysis. The procedure for the examination of the glands is not yet developed and will be amended to the final report at a later date. Other pup parameters like sex ratio, clinical observations, organ weights and gross necropsy findings did not reveal any treatment-related effects. The observed effects on absolute brain weights were secondary to the lower terminal pup body weights. Whereas the effect on sexual maturation of $\mathbf{F_1}$, $\mathbf{F_2}$ female and low-dose $\mathbf{F_2}$ male pups was minor, within the historical control, the effect on mid- and high-dose $\mathbf{F_1}$ and $\mathbf{F_2}$ male pups was outside the historical control data.

No changes in clinical chemistry or hematology were observed in parental animals when compared to controls. Histopathological examination revealed, as observed in other BAS 610 F studies, the adrenal gland as the target organ. This was evident by a treatment-related increase of absolute and relative adrenal gland weights in high-dose $\mathbf{F_1}$ males and high-dose $\mathbf{F_2}$ animals of both sexes, a dose- and treatment related increase of cytoplasmic vacuoles in the adrenal cortex at high-dose in $\mathbf{F_0}$ animals from both sexes and from mid-dose on in $\mathbf{F_1}$ animals from both sexes. The increased occurrence of vacuolation was considered to be non-adverse as the severity of vacuolation was in most affected animals only minimal or slight and as no other histopathological findings as cellular degeneration was observed.

Based on the effects on body weight development and food consumption during gestation the parental NOAEL in this study was identified at 750 ppm (83 mg/kg bw). In absence of any effects on fertility and reproductive performance the reproductive NOAEL was at least 1500 ppm (156 mg/kg bw). The developmental NOAEL in females was identified at 750 ppm (85 mg/kg bw) based on reduced pre-weaning pup body weights at 1500 ppm. In the male progeny, the NOAEL for developmental toxicity is 300 ppm (32 mg/kg bw/d) due to the delay in the onset of puberty that occurred beginning at 750 ppm (82 mg/kg bw/d).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 610 F

Description:	solid, granulate / light cream
Lot/Batch #:	COD-001260
Purity:	97.8% (tolerance +- 1.0%)
Stability of test compound:	The test substance was stable over the study period; (Expiry date: 30.07.2013)

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; 35 ± 1 days at beginning of treatment F_2 parental females as mating partners for selected F_2 parental males: 14-weeks at the day of delivery; 15-weeks at the beginning of mating.
Weight at dosing:	♂: 96.1 g- 123.2 g, ♀: 80.9g - 101.2 g
Source:	Charles River Laboratories, Sulzfeld, Germany,
Acclimation period:	about 7 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); bedding: Lignocel FS14 fibres supplied by SSNIFF Labordiaeten GmbH, Soest, Germany

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: 05-Jul-2011 - 18-Sep-2013
(In-life dates: 12-Jul-2011 (start of administration of F_0 parental animals) to 28-Jul-2012 (sacrifice of F_2 females)

2. Animal assignment and treatment:

BAS 610 F was administered in the diet to groups of 25 male and 25 female rats at nominal dose levels of 0, 300 (low dose), 750 (mid dose), and 1500 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. About 16 hours prior to sacrifice food was withdrawn.
At least 75 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.
After weaning, 25 male and 25 female F_1 pups of each treatment group were randomly selected as F_1 generation parental animals. It was attempted to take each litter into account. If fewer than 25 litters in these groups were available for selection or if one sex was missing in a litter, more animals were taken from different litters from the relevant test group to obtain the required number of animals.
All selected animals were treated with the test substance at the same dose level as their parents from post-weaning through adulthood up to about one day before they were sacrificed. At least 75 days after assignment of the F_1 generation parental animals, the males and females were mated overnight. The partners were randomly assigned so that matings of siblings were avoided.
Like F_0 females, F_1 females were allowed to litter and rear their pups (F_2 generation pups) until day 4 (standardization) or 21 after parturition. Shortly after weaning of F_2 pups, the F_1 parental animals were sacrificed.

At least 75 days after assignment of the F_2 generation parental animals, 10 control and 10 high dose males and 20 un-treated females were mated overnight. The 20 female rats (to produce the F_3 litter) were 14 weeks old when they arrived from the breeder and were mated after an acclimatization period of about 5 days The partners were randomly assigned so that matings of siblings were avoided.
Like $F_{0/1}$ females, F_2 females were allowed to litter and rear their pups (F_3 generation pups) until day 4 (standardization) or 21 after parturition. Shortly after weaning of F_3 pups, the F_2 parental animals were sacrificed.
With the exception of F_1 and F_2 generation pups, which were chosen as F_1 and F_2 generation parental animals, respectively, all pups were sacrificed by means of CO_2 after standardization or weaning.

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. Diet preparations were performed at intervals, which guaranteed that the test substance in the diet remained stable throughout the feeding period.

The following nominal dose levels were selected for the study:

300	ppm as low dose
750	ppm as intermediate dose
1500	ppm as high dose

BAS 610 F concentrations in the diet of the F_0, F_1 and F_2 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 BAS 610 F 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:

$$IT_x = \frac{FC_x \times C}{BW_y}$$

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 BAS 610 F 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 35 days

Homogeneity and concentration control analyses were carried out at the beginning and end of the premating phase, during the gestation (except in the F1 females) and lactation periods and towards the end of the F3 generation. According to the SOP, for homogeneity analysis three randomly sampled specimen from the top, middle and bottom of the storage containers were sampled and analyzed.

Table 5.6.1-1: 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 [%]
300	08.07.2011	341.334			
300	08.07.2011	340.434	113.6		
300	08.07.2011	334.243			
300	08.07.2011	331.465	111.0		
300	08.07.2011	324.214			
300	08.07.2011	321.286	107.6	110.7	2.7
750	08.07.2011	831.236			
750	08.07.2011	824.822	110.4	110.4	
1500	08.07.2011	1510.888			
1500	08.07.2011	1498.895	100.3		
1500	08.07.2011	1506.078			
1500	08.07.2011	1499.809	100.2		
1500	08.07.2011	1304.348			
1500	08.07.2011	1455.880	92.0	97.5	4.9
300 R	23.09.2011	319.844	106.6		
300 R	23.09.2011	345.092	115.0		
300 R	23.09.2011	306.407	102.1	107.9	6.1
750 R	23.09.2011	893.605	119.1		
1500 R	23.09.2011	1558.102	103.9		
1500 R	23.09.2011	1568.461	104.6		
1500 R	23.09.2011	1565.548	104.4	104.3	0.3
150 R	14.10.2011	142.258	94.8		
150 R	14.10.2011	131.790	87.9		
150 R	14.10.2011	131.028	87.4	90.0	4.6
375 R	14.10.2011	395.829	105.6		
750 R	14.10.2011	806.484	107.5		
750 R	14.10.2011	829.891	110.7		
750 R	14.10.2011	826.591	110.2	109.5	1.5
300	11.11.2011	353.849	117.9		
300	11.11.2011	341.115	113.7		
300	11.11.2011	318.755	106.3	112.6	5.3
750	11.11.2011	899.598	119.9		
1500	11.11.2011	1634.699	109.0		
1500	11.11.2011	1597.751	106.5		
1500	11.11.2011	1558.335	103.9	106.5	2.4
300	20.01.2012	326.958	109.0		
300	20.01.2012	378.640	126.2		
300	20.01.2012	355.936	118.6	117.9	7.3
750	20.01.2012	756.210	100.8		
1500	20.01.2012	1500.772	100.1		
1500	20.01.2012	1510.648	100.7		
1500	20.01.2012	1598.763	106.6	102.4	3.5

R Reserve samples; # based on mean values of the three individual samples
Values may not calculate exactly due to rounding of values

Table 5.6.1-1: continued

Nominal Dose level [ppm]	Sampling	Concentration [ppm]	Nominal concentration [%]	Mean nominal concentration [%]#	Relative standard deviation [%]
150	24.02.2012	162.498	108.3		
150	24.02.2012	157.568	105.0		
150	24.02.2012	163.991	109.3	107.6	2.1
375	24.02.2012	407.304	108.6		
750	24.02.2012	756.601	100.9		
750	24.02.2012	772.720	103.0		
750	24.02.2012	811.195	108.2	104.0	3.6
1500	01.07.2012	1523.399	101.6		
1500	01.07.2012	1653.240	110.2		
1500	01.07.2012	1685.746	112.4	108.1	5.3

R Reserve samples; # based on mean values of the three individual samples
Values may not calculate exactly due to rounding of values

Relative standard deviations of the homogeneity samples in the range of 0.3 to 7.3% indicate the homogenous distribution of BAS 610 F in the diet preparations. The actual test-item concentrations were in the range of 90.0 to 117.9% of the nominal concentrations. The mean found concentrations are above the specification limit of 110 %, but mean found concentrations within ± 15 % of the target concentration can be regarded acceptable for complex matrices like diet.

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), estrous cycle duration, number of mating days, duration of gestation, number of implantation sites, postimplantation loss and % postimplantation loss, number of pups delivered per litter, duration of sexual maturation (days to vaginal opening, days to preputial separation), anogenital distance, anogenital index	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, sexual maturation data (vaginal opening, prepu¬tial separation), males with a certain amount of abnormal sperm (cutoff value: 0.9-quantile [90%] of control groups	Pairwise comparison of each dose group with the control group using FISHER'S EXACT test for the hypothesis of equal proportions
Presence of areolae/nipples, 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
Pup organ weights (absolute and relative)	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 (two-sided) for the 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 For parameters with unidirectional changes: Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians
Spermanalysis parameters	Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) with Bonferroni-Holm adjustment for the hypothesis of equal medians For the percentage of abnormal sperms (ABNORMAL6_C) values < 6 % were set to 6 % (cut off 6 %)

Statistics of pathology

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.
DOFC (differential ovarian follicular count)	Pair wise comparison of the high dose group with the control group using the WILCOXON-test (one-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.

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, F_1 and F_2 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 retro-orbital venous plexus from fasted animals following isoflurane anesthesia.
The examinations were carried out a few days before terminal sacrifice of the animals in 12 randomly selected animals per test group and sex of the F_0, F_1 and F_2 parental generation. The following hematological and clinical chemistry parameters were determined:

Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
	Chloride	✓	Bilirubin (total)	✓	Aspartate aminotransferase (AST)
	Magnesium	✓	Cholesterol	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Creatinine	✓	γ-glutamyl transpeptidase (γ-GT)
	Potassium	✓	Globulin (by calculation)		
	Sodium	✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		
Hematology:					
✓	Leukocytes	✓	Reticulocytes		
✓	Erythrocytes	✓	Differential blood count		
✓	Hemoglobin	✓	Mean corpuscular volume		
✓	Hematocrit (calculation)	✓	Mean corpuscular hemoglobin (calculation)		
✓	Platelets	✓	Mean corp. hemoglobin conc. (calculation		

6. Estrous cyle determination:

Estrous cycle length was evaluated by daily analysis of vaginal smear for all F_0 and F_1 female parental rats for a minimum of 3 weeks prior to mating and was continued throughout the mating period until the female exhibited evidence of copulation. Moreover, at necropsy a vaginal smear was examined to determine the stage of the estrous cycle for each F_0 and F_1 female with scheduled sacrifice.

7. Male reproduction data

For the males, mating and fertility indices were calculated for F_1 and F_2 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

8. Sperm parameters

Immediately after necropsy and organ weight determination the right testis and cauda epididymis were taken from the F_0 and F_1 males of all dose groups. The following parameters were determined:

- sperm motility
- sperm morphology
- sperm head count (cauda epididymis)
- sperm head count (testis)

Sperm motility examinations and the preparations of the specimens for sperm morphology were carried out in a randomized sequence. Sperm morphology and sperm head count (cauda epididymis and testis) were evaluated for the control and highest dose group, only. Sperm motility was investigated by microscopic evaluation. Sperm morphology was evaluated microscopically after vital staining with eosin. Sperm head counts in cauda eoididymis and testes were determined microscopically after homogenization using a MAKLER chamber.

9. Female reproduction and delivery data

For the females, mating, fertility and gestation indices were calculated for F_1, F_2, and F_3 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 and F_2 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.

$$\text{Postimplantation loss [\%]} = \frac{\text{number of implantations} - \text{number of pups delivered}}{\text{number of implantations}} \times 100$$

10. Litter data

All F_1, F_2 and F_3 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 and F_2 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:

$$\text{Sex ratio [\%]} = \frac{\text{number of live male or female pups on day 0 / 21}}{\text{number of live male and female pups on day 0 / 21}} \times 100$$

Sexual maturation was determined in F_1 and F_2 pups selected as parental animals. Females were evaluated daily for vaginal opening with examinations initiating on day PND 27. On the day of vaginal opening the body weights of the respective animals were determined. Males were evaluated daily for preputial separation with examinations initiating on day PND 38. On the day of preputial separation the body weights of the respective animals were determined. An analysis was performed to graphically compare the ages and weights at puberty of the individual animals which were selected for further breeding with the average growth progression of all F_1 and F_2 control animals, using the change in body weight as a marker for general animal development In addition, male pups were examined for the presence of nipple/areola anlagen on PND 12 and were re-examined on PND 20 or PND 21.

11. Sacrifice and pathology:

All F_0 and F_1 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.

Pathology:

The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: All animals of control and high dose groups; male animals suspected of impaired fertility and female animals that were non-pregnant of low and mid dose groups; all low and mid dose animals which died intercurrently of were sacrificed moribund).

C	W	H		C	W	H		C	W	H	
✓	✓	✓	adrenals	✓	✓		liver	✓	✓		spleen
✓	✓		brain	✓			mandibles‡	✓	✓	#	testes§$
	✓		cauda epidiymes	✓	✓	#	ovaries$	✓	✓		thyroid w. parathyroid gland
✓		#	coagulation glands	✓		#	oviducts	✓	✓	#	uterus (& cervix uteri)
✓	✓	#	Epididymides§$	✓	✓	#	pituitary	✓		#	vagina
✓		✓	gross lesions	✓	✓	#	prostate		✓		body (anesthethized)
✓	✓		kidneys	✓	✓	#	seminal vesicles[a]				

§ left of paired organs sampled and histopathologically examined; $ fixed in Bouin's solution for animals killed as scheduled, for animals that died intercurrently the mentioned organs were fixed in neutral buffered 4% formaldehyde; [a] with coagulation glands; ‡ the mandibles were cut longitudinally into two halfs between the incisivi; the left halfs of the mandibles were fixed in 4% formaldehyde solution, the right halfs were stored deep-frozen (-20°C) individually.

The organs or tissues were fixed in neutral buffered 4% formaldehyde or in BOUIN's solution. The hematoxylin-eosin (HE) stained slides were examined and assessed by light microscopy.

For F_1 maternal animals differential ovarian follicle count (DOFC), sections were prepared with 2 µm thickness and every 50 µm a serial section was taken and mounted on glass slides. Primordial follicles and growing follicles were counted by light microscopy. In order to prevent multiple counting - especially of the growing follicles - only follicles with an oocyte with visible chromation on the slide were counted.

In addition, special histopathological investigation of mammary fatpads from 20 F_2 parental males, using a whole-mount staining technique, was performed. Instead of asservating both the skin and the mammary fatpad together, only the underlying fatpads in the area of the mammary line was sampled. Using forceps, the fatpads were spread onto microscope slide and fixed in Carnoy's fixative and stained in Carmine-Alum. Finally, glands were cleared in xylene and the cleared tissue photographed under microscopy. For archivation, fatpads were embedded in paraplast according to standard operating procedures and stored.
The procedure for the examination of the photographs is not yet developed. Therefore these results will be amended to the final report at a later date.

With the exception of those F_1 and F_2 generation pups, which were chosen as F_1 and F_2 generation parental animals, respectively, all pups were sacrificed by means of CO_2 after standardization or weaning. All culled pups, including stillborn pups and those that died during their rearing period, and pups killed after weaning were subjected to a macroscopic (external and visceral) examination. Brain, spleen and thymus weights were taken from the first male and the first female pup/litter for pups killed at PND 21 (weaning) (except F_3 pups). Relative organ weights were calculated on PND 21 pup weights.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

See Section B 3. above

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 and female **F_0**, **F_1** and **F_2** parental animals.

Clinical observation of **F_0** female animals revealed a palpable mass at the throat as well as a skin lesion at the shoulders (both sides) and throat in one control female (#122). Two sperm positive **F_0** control dams (#106, #114), two sperm positive low dose dams (#128, #133) and one sperm positive high dose dam (#194) did not deliver **F_1** pups. The absence of a dose-response indicated a spontaneous origin of these findings.

Clinical observation of **F_1** male animals revealed a palpable mass at the throat in one control male (#215). One mid dose dam delivered all stillborn pups, had undelivered pups palpable in the abdomen from PND 0-1 and had no more palpable pups in the abdomen on PND 2 (#362). The low incidence, within the historical control range of the performing lab, and the absence of a dose-response relationship indicated a spontaneous origin of this finding. Two sperm positive **F_1** control dams (#303, #306), one sperm positive low dose dam (#333) and one sperm positive high dose dam (#395) did not deliver **F_2** pups. The absence of a dose-response indicated a spontaneous origin of these findings.

2. Mortality

No treatment-related or spontaneous mortality was observed throughout the study.

C. PARENTAL BODY WEIGHT AND BODY WEIGHT GAIN

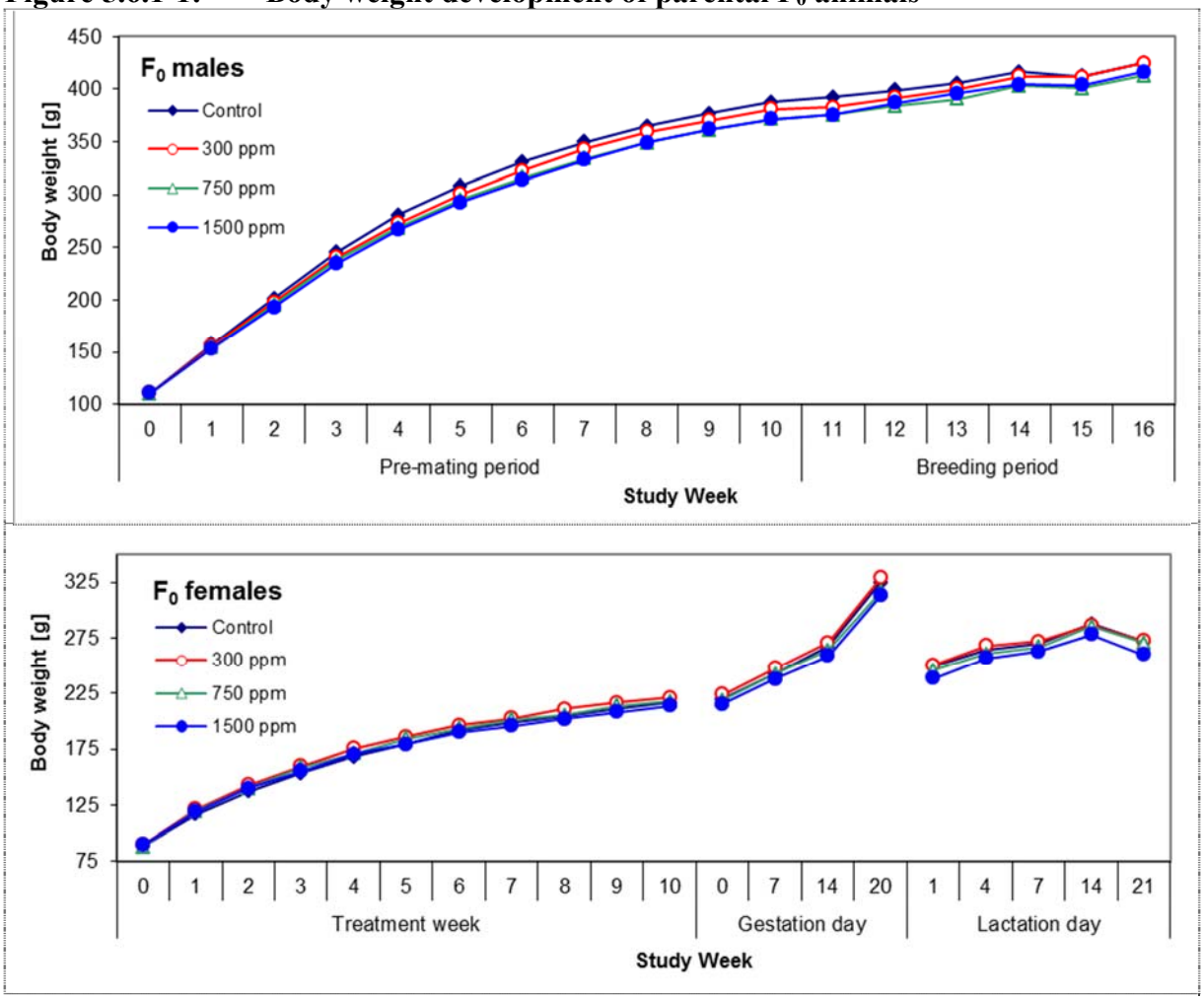
Body weight development was impaired in high dose parental **F_0** and **F_1** females during gestation and **F_2** males during premating.

F_0 parental animals:
The body weights of the parental males were considered comparable to the concurrent control group throughout the entire study period as was the body weight change.

Body weights and body weight change of high-dose females were generally lower than controls after week 6 and 4 respectively of the premating period and throughout gestation. The reduction in body weight gain achieved statistical significance between GD 7-14 (about 16% below the concurrent control values) [Figure 5.6.1-1]. High-dose parental females also had statistically significantly lower body weights on PND1 and PND 21 (about 4% respectively below the concurrent control values).

The body weights of the mid- and low-dose parental females were comparable to the concurrent control group as were the body weight change throughout the study duration [Figure 5.6.1-1].

Figure 5.6.1-1: Body weight development of parental F_0 animals



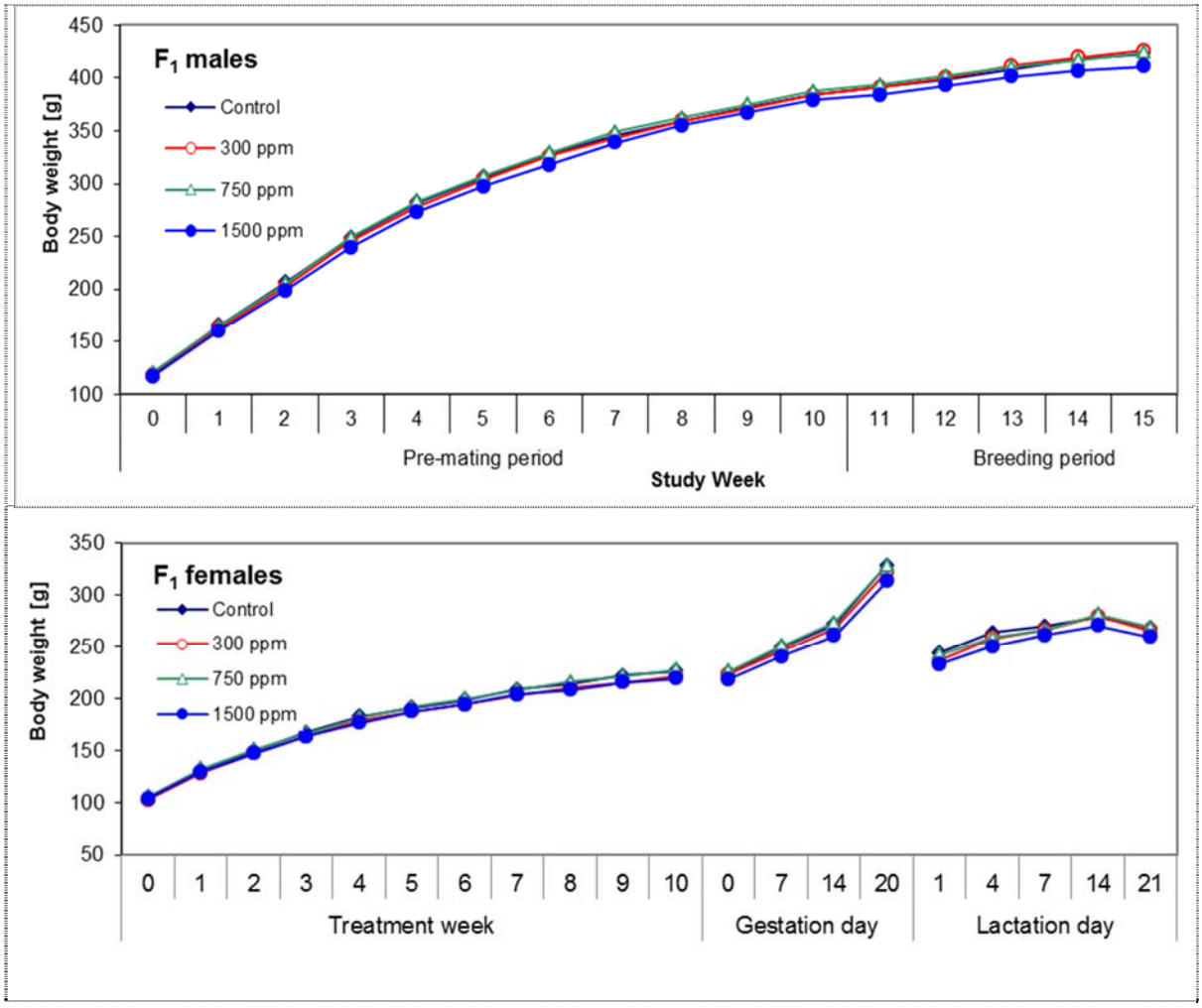
Body weight of high-dose **F_0** males was statistically significantly lower in weeks 2-6 (up to 5% below the concurrent control values) [Figure 5.6.1-1]. In addition, body weight change was statistically significantly decreased during study weeks 0-2 (up to 9% below the control). These changes are considered secondary to reduced food consumption during this timeframe and not considered a sign of general toxicity. The statistically significantly increased body weight change in the high-dose males during study weeks 11 - 12 was considered to be spontaneous in nature. The body weight change of the mid-dose males was statistically significantly decreased during study weeks 0 - 1 (about 6% below the control). As this nominal solitary reduction in body weight change had no statistically significant correlate in reduced body weight in this dose group during this period, it was considered to be spontaneous and unrelated to treatment.

F_1 parental animals:
Body weights and body weight change of all treated **F_1** males were considered comparable to the concurrent control during the entire study period [Figure 5.6.1-2].

High-dose F_1 females had significantly lower body weights on gestation day 20 (about 5% below the concurrent control values). This corresponded to a lower body weight gain during gestation (about 9% below the control values) [Figure 5.6.1-2].

Body weights and body weight change of all treated **F_1** females were considered comparable to the concurrent control during the entire premating and lactation period. In addition, the body weights and body weight change of the mid- and low-dose females were considered comparable to the concurrent control group throughout the entire gestation period.

Figure 5.6.1-2: Body weight development of parental F_1 animals



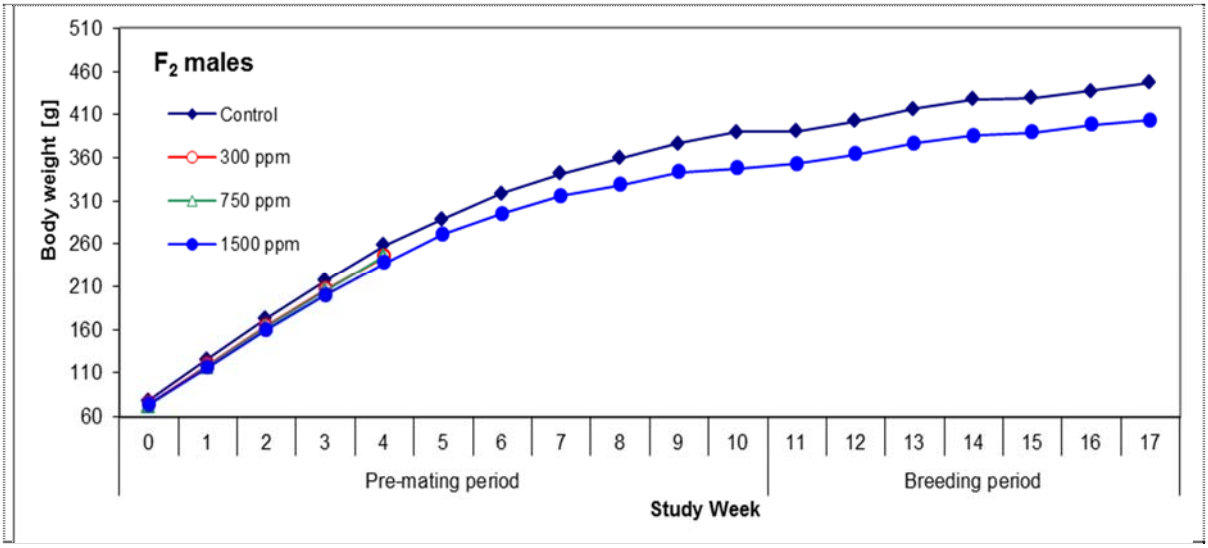
High-dose **F_1** males had a significantly lower body weight change during study weeks 13-14, that was considered to be incidental as where the statistically lower body weight in the high-dose females **F_1** on PND 4 as well as the significantly lower body weight change in mid-dose females during premating weeks 2-3.

F_2 parental animals:

High-dose **F_2** males body weights were significantly below the concurrent control values during study weeks 1-4 and 6-17 (up to 8% and 11%, respectively). In addition, body weight change was statistically significantly below the concurrent control values during several parts of the study (up to 62%). For the mid-dose and low-dose **F_2** males body weights were statistically significantly below the concurrent control values during study weeks 1-4 and 3-4 (up to 7% and 5% respectively). In addition, body weight change was significantly below the concurrent control values during study weeks 0-1 (mid-dose, about 6%) and 0-1, 0-4 (low dose, about 6% and 5%).

The mean body weights and body weight change of all test substance treated **F_2** females were comparable to the concurrent control group throughout the entire study period.

Figure 5.6.1-3: Body weight development of parental F_2 animals



D. PARENTAL FOOD CONSUMPTION AND COMPOUND INTAKE

In all dose groups and generations, both sexes, intermittent reductions of food consumption were noted, either during premating, gestation and lactation phases of this study. The effects were consistent in the high-dose group **F_0** and **F_1** females, where the reduction, whether statistically significant or insignificant, was notable during the entire premating and gestation periods.

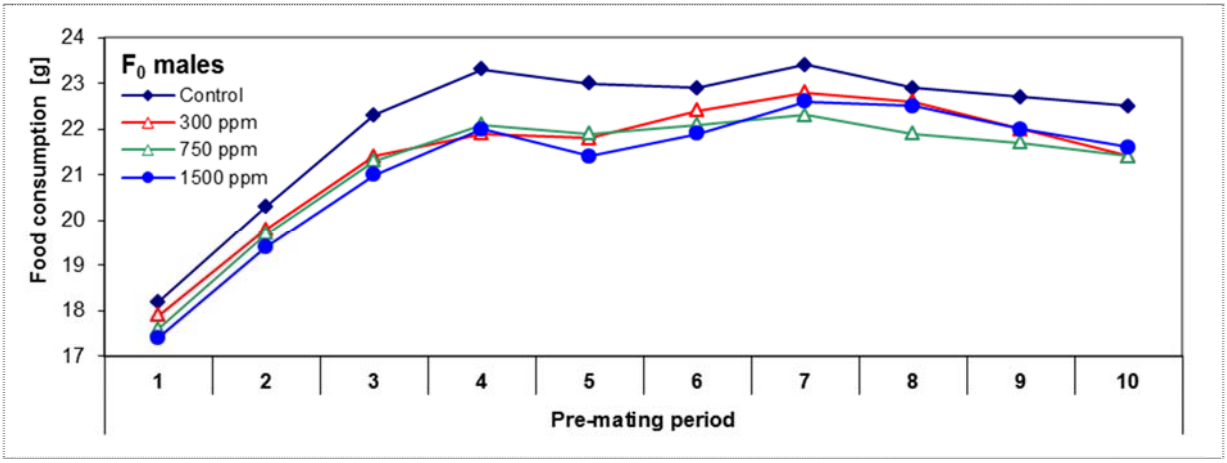
F_0 parental animals:

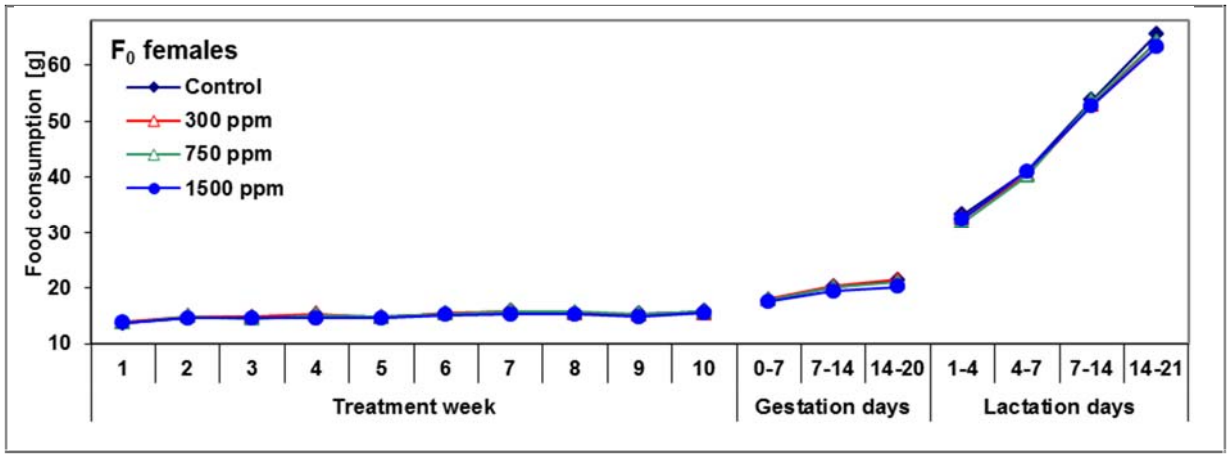
A statistically significant decrease of food consumption by up to 7% was observed in high-dose **F_0** males during premating weeks 0-5. In addition, significantly reduced food consumption of mid- and low-dose males up to 5% and 6% was observed during premating weeks 2-4 (mid dose) and 3-4 (low dose), respectively.

Food consumption of all high-dose **F_0** females was slightly but non-significantly reduced beginning in week 3 of the premating phase and continuing throughout gestation. However, during lactation the food consumption of these animals was comparable to that of the concurrent controls.

Food consumption of all mid- and low-dose **F_0** females was comparable to the concurrent control values during the entire study period.

Figure 5.6.1-4: Food consumption of parental F_0 animals





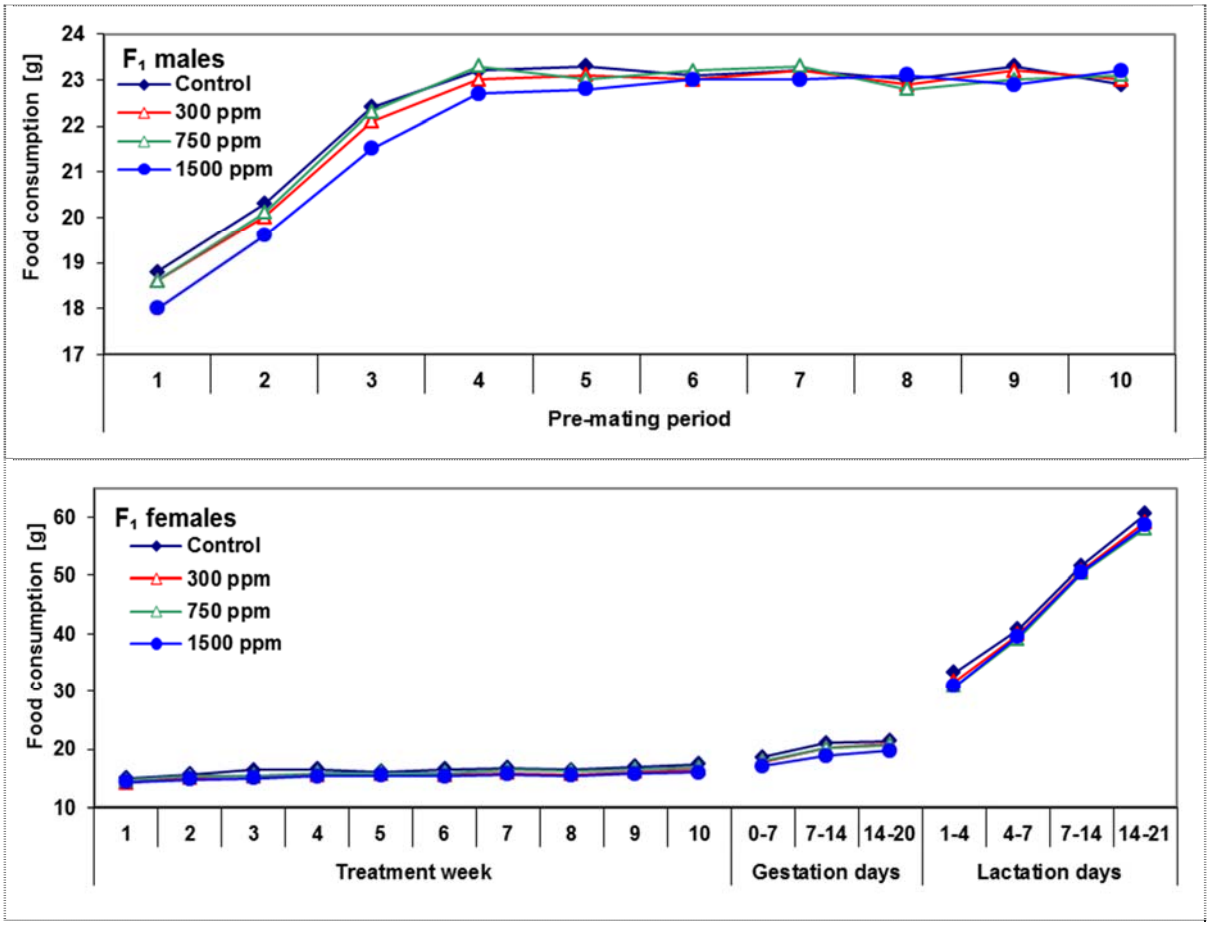
F_1 parental animals:

Food consumption of all test substance-treated F_1 males was comparable to the concurrent control values during the entire study period.

Food consumption of high-dose **F_1** females was reduced up to 9% throughout the entire premating period and up to 10% during the whole gestation period [Figure 5.6.1-5]. The difference was significant during premating weeks 1-4 and 5–10. No changes in food consumption of the high-dose females were noted during lactation.

Food consumption of mid- and low-dose **F_1** females was comparable to the concurrent control throughout the entire gestation and lactation period.

Figure 5.6.1-5: Food consumption of parental F_1 animals



Food consumption of mid-dose **F_1** females was about 5% below control values during premating weeks 2-3. For the low-dosed **F_1** females the food consumption was also significantly below the control values during premating weeks 0-1; 2-3 and 5-7 (5%; 7% and 5%, respectively). These temporary, inconsistent changes were not regarded to be related to treatment.

F_2 parental animals:

A statistically significant reduction in food consumption was observed in high-dose **F_2** males during study weeks 0 - 4 and 9 - 10 (up to 9% and 8%, respectively) as well as in mid-dose and low-dose **F_2** males during study weeks 0 - 4 and 1 - 4 (up to 7% and 6% respectively) [Figure 5.6.1-6]. For the mid-dose and low-dose F_2 males food consumption was statistically significantly below the concurrent control values during study weeks 0 - 4 and 1 - 4 (up to 7% and 6% respectively).

Food consumption of high-dose **F_2** females was significantly below the concurrent control during study weeks 1 - 2 (about 7%). No changes in food consumption were noted for the mid-dose and low-dose females during the study period. No changes in food consumption were observed for all females during gestation and lactation period.

Figure 5.6.1-6: Food consumption of parental F_2 animals

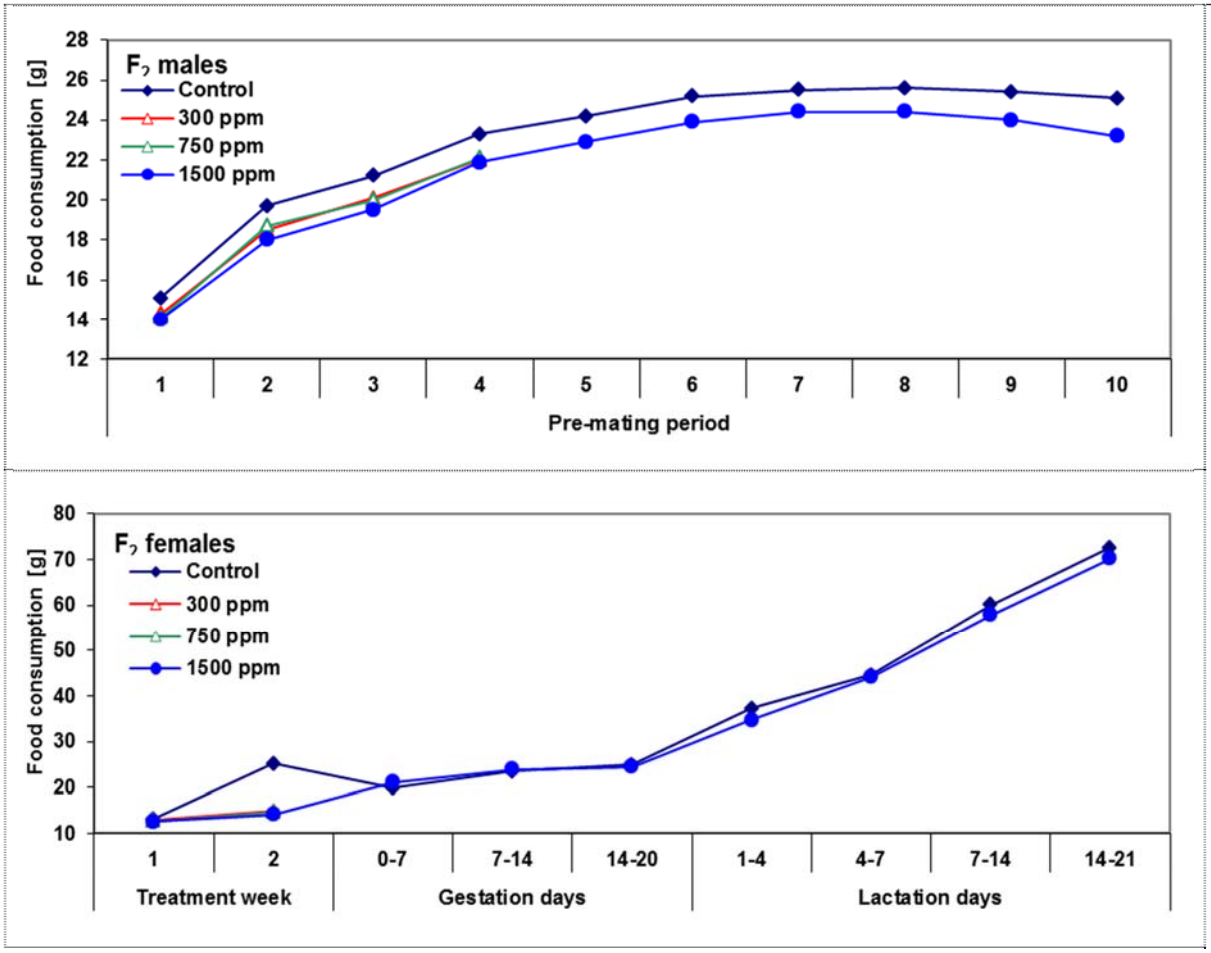


Table 5.6.1-2: Average BAS 610 F intake (mg/kg bw/d) in parental animals

Dose	300/150 ppm		750/375 ppm		1500/750 ppm	
Group & sex	average	min/max	average	min/max	average	min/max
F_0 males[a]	26.9	17.3 / 48.4	67.8	44.3 / 120.3	135.6	89.8 / 235.5
F_1 males[a]	27.3	18.7 / 47.9	67.4	46.3 / 115.8	136.5	94.8 / 232.7
F_2 males	43.4[b]	32.1 / 58.1	109.7[b]	80.7 / 145.8	159.3[a]	101.9 / 284.2
F_0 females[a] (premating)	28.4	21.5 / 46.6	71.8	55.1 / 117.1	142.5	109.8 / 229.6
F_0 females[c] (gestation)	24.2	23.8 / 24.6	61.0	59.5 / 62.2	120.5	117.4 / 122.4
F_0 females[d] (lactation*)	26.2	19.5 / 33.5	66.6	48.4 / 84.4	135.8	102.0 / 170.7
F_1 females[a] (premating)	28.3	23.0 / 42.1	70.3	57.4 / 104.4	138.7	111.2 / 207.3
F_1 females[c] (gestation)	24.0	23.4/ 24.6	58.9	56.7 / 60.3	116.0	112.9 / 117.7
F_1 females[d] (lactation*)	25.9	20.1 / 31.8	63.1	47.7 / 77.2	131.6	99.4 / 162.7
F_2 females[e]	49.5	42.9 / 56.1	124.7	107.6 / 141.8	243.8	210.1 / 277.5

[a] week 0-10 [b] week 0-4 [c] Days 0 – 20 [d] Days 1 – 21 [e] week 0-2

E. ESTROUS CYCLE DETERMINATIONS

Estrous cycles as determined during 3 week prior to mating were very regular in all dose groups in **F_0** and **F_1** females. The mean cycle length was 4.2, 4.2, 4.1 and 4.2 days in **F_0** and 4.4, 4.1, 4.1 and 4.0 days in **F_1** females at 0, 300, 750, and 1500 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.

F_0 males mated except one high-dose male (#85) and one mid-dose male (#64) and induced pregnancy in 25, 25, 24 and 24 females at 0, 300, 750 and 1500 ppm, respectively [see Table 5.6.1-3]. All **F_1** males and nearly all **F_2** males, except male #421 mated.

Table 5.6.1-3: Reproduction parameters of male rats treated with BAS 610 F

Parental generation		F_0				F_1				F_2	
Dose	[ppm]	0	300	750	1500	0	300	750	1500	0	1500
Animals per dose		25	25	25	25	25	25	25	25	10	10
Male fertility											
- placed with females		25	25	25	25	25	25	25	25	10	10
- mated	[n]	25	25	24	24	25	25	25	25	9	10
- mating index	**[%]**	**100**	**100**	**96**	**96**	**100**	**100**	**100**	**100**	**90**	**100**
- pregnant	[n]	23	23	24	23	23	24	24	24	9	10
- Fertility index	**[%]**	**92**	**92**	**96**	**92**	**92**	**96**	**96**	**96**	**90**	**100**

None of the **F_0** and **F_2** male mating partners that failed to mate displayed histopathological findings that could explain infertility. Low-dose **F_0** male #33 showed a moderately reduced size of the testes and epididymides and correspondingly a severe multifocal tubular degeneration in the left testicle resulting in an aspermia of epididymides. These findings may explain the infertility of female mating partner #133.

2. Sperm analysis

Sperm analysis (number of homogenisation resistand sperm heads in testes and cauda eypidymidis, % sperm motility and abnormal sperm) did not indicate any effects of treatment in F_0 males and revealed a slight increase in abnormal sperm in F_1 males [see Table 5.6.1-4].

Table 5.6.1-4: Sperm parameters of males administered BAS 610 F

Parental generation		F_0				F_1			
Dose	[ppm]	0	300	750	1500	0	300	750	1500
Sperm count	[10^6 / g]								
Testis		107			99	108			105
Cauda epididymis		527			531	534			574
Sperm motility	[%]	86	86	87	87	89	88	87	88
Normal sperm	[%]	92.8			92.6	93.3	92.6	92.1	92.0
Abnormal sperm	[%]	7.2			7.4	6.7	**7.4***	**7.9***	**8.0***
Range abnormal sperm	[%]					2.5 - 14	2 - 12	1 – 20	3.5 – 24
Animals exceeding historical control range	[n]					1	3	1	2
Animals exceeding study control range	[n]					-	0	1	1

Note: Historical control range (97.5th percentile): 0-11%

The dose-dependent increase of the percentage of abnormal sperms in **F_1** males was marginal (6.7 % abnormal sperms in the controls versus 8.0 % abnormal sperms in the high-dose group). Most of the abnormalities comprised breakage of the sperms in heads and tails as well as smaller heads. On an individual animal basis, the highest percentage of abnormal sperm in the control group was 14% (#221) (range 2.5-14%). There was no animal in the low-dose group exceeding the incidence of the concurrent control range. In the mid-dose group one animal exceed the incidence of the concurrent control group (#255, 20%) as well as the 97.5th percentile of the historical control range (0-11%). However, within the historical controls, a single finding of 22% abnormal sperm has been observed (study report PART III, Supplement). There were no effects on mating or reproduction from male #255 mated with female #371. At the highest dose tested, one animal was at the upper range of the concurrent control incidence (#295, 14.5%) and one animal exceeded the historical control incidence (#285, 24%). There were no effects on mating or reproduction from male #285 mated with female #391.

Based on two single findings of increased abnormal sperm in the mid- and high-dose treatment groups, that did not affect mating or reproduction of those animals, the findings were regarded as incidental and not adverse.

3. Female reproductive performance

Female reproductive performance was not affected by treatment.

All control and low-dose **F_0** females mated as well as 24 out of 25 females at the mid- and high-dose treatment levels. The numbers of females becoming pregnant was 23, 23, 24 and 23 at 0, 300, 750 and 1500 ppm, respectively [Table 5.6.1-5]. Control females #106 (mated with control male #6), #114 (mated with control male #14), low-dose females #128 (mated with male #28), #133 (mated with male #33), mid-dose female #164 (mated with male #64) and high-dose females #185 (mated with male #85), #194 (mated with male #94) did not become pregnant. Neither of the non-pregnant **F_0** females (#106, #114, #128, #133, #164, #185, #194) nor their male mating partners (#6, #14, #28, #33, #64, #85, #94) showed gross- or histopathological-lesions explaining the apparent infertility.

All **F_1** females mated and 23, 24, 24 and 24 females became pregnant at 0, 300, 750 and 1500 ppm, respectively [Table 5.6.1-5]. Control females #303 (mated with male #223), #306 (mated with male #220), low-dose female #333 (mated with male #243), mid-dose female #372 (mated with #254) and high-dose female #395 (mated with male #281) did not become pregnant. For the low-dose and mid-dose females histopathological examination revealed possible explanations for the apparent infertility. In low-dose female #333 a dilation of the uterus with suppurative contents and in the non-pregnant mid-dose female #372, a moderate dilation of the uterus was observed and confirmed histopathologically. During estrus cycle, luminal dilation of the uterus is commonly noted during the proestrus. Because both females were in the metestrus, the dilation of the uterus was interpreted as sign of asynchrony in these females that might be responsible for the infertility. Neither of the remaining non-pregnant **F_1** females (#306, #333, #395) nor any of the **F_1** male mating partners (#223, #220, #243, #254, #281) showed gross- or histopathological-lesions explaining the apparent infertility.

All **F_2** females with the exception of control female #606 (mated with control male #421) mated and 9 and 10 females became pregnant at 0 and 1500 ppm respectively. No histopathological examination was performed to assess the apparent infertility of control female #606 or control male #421.

The pre-coital interval was in the range of 2.0 to 3.2 days in **F_0** females, of 2.1 to 2.8 days in **F_1** females and of 1.9 to 2.1 days in **F_2** females and displayed no relation to treatment level. Likewise, duration of gestation was similar in **F_0** (21.8 to 22.0 days), **F_1** females (21.9 to 22.3 days) and **F_2** females (22.0 days) [see [Table 5.6.1-5].

There was no significant effect of treatment on gestational parameters in female **F_0**, **F_1** and **F_2** generation.
There was no statistically or biologically significant difference in the number of implantation sites.

The post-implantation loss was comparable between control and treated groups for the **F_0** (mean % per litter: 2.7 to 10.3%), **F_1** generation females (mean % per litter: 4.1 to 6.4%) and **F_2** generation females (mean % per litter: 3.4 to 9.7%). The incidences displayed no dose-response relationship and were within the historical control range.

The gestation index in **F_0** female ranged between 100 and 96% without any relation to treatment. **F_0** mid-dose female #167 had all its implants resorbed, and displayed dilatation of the renal pelvis. These findings were considered to be independent from each other and unrelated to treatment.

The gestation index in **F_1** females ranged between 100% and 96% in the mid dose without any relation to treatment [see Table 5.6.1-5].

The gestation index in **F_2** females ranged between 100% and 90% in controls without any relation to treatment.

Mean litter sizes between 11.7 and 12.0 in the **F_0** generation, between 11.6 and 12.0 in the **F_2** generation and between 11.2 and 12.4 in the **F_3** generation were without any relation to dose [see Table 5.6.1-5].

Then number of stillborn **F_1** and **F_3** pups was comparable between control and treated groups and significantly increased in the **F_2** mid-dose treatment group, due to female #362 delivering all 10 pups stillborn. When the stillbirths arising from this single dam are excluded from the results, the number of stillborn pups is comparable between the groups and well-within the historical control range. In addition, the incidence of one total litter loss is within the historical control range. Therefore the increase in the number of stillborn pups was considered to be spontaneous in nature and not treatment-related.

There was no effect on the live birth index. It ranged from 99 to 100% in **F_1** pups, from 96 to 100% in **F_2** pups and from 98 to 100% in **F_3** pups. The values were well within the range of biological variation (95 to 100%).

Table 5.6.1-5: Reproduction and gestational parameters of female rats treated with BAS 610 F

Parental generation		F_0				F_1				F_2	
Dose	[ppm]	0	300	750	1500	0	300	750	1500	0	1500
Animals per dose		25	25	25	25	25	25	25	25	10	10
Female fertility											
- placed with males		25	25	25	25	25	25	25	25	10	10
- mated	[n]	25	25	24	24	25	25	25	25	9	10
- mating index	[%]	100	100	96	96	100	100	100	100	90	100
- pregnant	[n]	23	23	24	23	23	24	24	24	9	10
- **Fertility index**	[%]	92	92	100	96	92	96	96	96	100	100
Pre coital interval	[days]	3.2	2.9	2.0	2.2	2.2	2.2	2.8	2.1	2.1	1.9
Duration of gestation	[days]	22.0	22.0	21.8	21.9	22.1	22.1	22.3	21.9	22.0	22.0
Implantation sites, total	[n]	285	288	300	279	288	299	302	290	116	123
- dto per dam	[n]	12.4	12.5	12.5	12.1	12.5	12.5	12.6	12.1	12.9	12.3
Post implantation loss	[n]	9	12	32	8	12	19	18	12	4	11
- dto per dam	[n]	0.4	0.5	1.3	0.3	0.5	0.8	0.8	0.5	0.4	1.1
- dto per litter	[mean %]	3.1	5.4	10.3	2.7	4.1	6.4	6.0	4.2	3.4	9.7
Females with liveborn		23	23	23	23	23	24	23	24	9	10
- **Gestation index**	[%]	100	100	96	100	100	100	96	100	100	100
- with stillborn pups	[n]	2	0	0	1	1	2	3	3	0	2
- with all stillborn	[n]	0	0	0	0	0	0	1	0	0	0
Pups delivered	[n]	276	276	268	271	276	280	284	278	112	112
- per dam	[mean n]	12.0	12.0	11.7	11.8	12.0	11.7	11.8	11.6	12.4	11.2
- liveborn	[n]	274	276	268	270	275	278	**272****	273	112	110
- stillborn	[n]	2	0	0	1	1	2	**12****[a]	5	0	2
- **Live birth index**	[%]	99	100	100	100	100	99	96	98	100	98

[a] 2 if all 10 stillborn pups of dam #362 are excluded

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnet-test two sided or Fisher's exact test one sided)

Values may not calclate exactly due to rounding of values

G. PUP DATA

1. Survival

Survival of pups was not treatment related affected in either generation.

The viability index (survival days 0 to 4 pre cull) ranged between 99% in **F_1** control and low dose pups and 100% in mid and high-dose pups [see Table 5.6.1-6].
In the F_2 animals, the viability index ranged between 100% in high-dose pups and 99% in control, low- and mid-dose pups. A single mid-dose female (#362) delivered all 10 pups stillborn. When the stillbirths arising from this single dam are excluded from the results, the number of stillborn pups is comparable between the groups and well-within the historical control range. Therefore the increase in the number of stillborn pups was considered to be spontaneous in nature and not treatment-related. For **F_3** animals, the viability index was 99% in control and the high-dose group.

The lactation index (survival day 4 post cull to 21) was not affected by treatment. As all **F_1** and **F_2** pups survived till weaning, the lactation index was 100% for all groups [see Table 5.6.1-6].

In **F_3** pups the lactation index ranged from 100% in high-dose pups to 99% in control pups.

Table 5.6.1-6: Pup survival, sex-ratio and body weights

Pup generation		F_1				F_2				F_3	
Dose	[ppm]	0	300	750	1500	0	300	750	1500	0	1500
Number of litters		23	23	23	23	23	24	24	24	9	10
- with liveborn pups		23	23	23	23	23	24	23	24	9	10
- with stillborn pups		0	0	0	0	1	2	3	3	0	2
Pups liveborn	[n]	274	276	268	270	275	278	**272****	273	112	112
Pups stillborn [a]	[n]	2	0	0	1	1	2	**12****	5	0	2
Pups died	[n]	4	0	0	0	0	1	3	0	0	0
Pups canibalized	[n]	0	2	1	1	3	3	0	0	2	1
Pups culled day 4	[n]	86	96	85	86	88	84	85	81	39	31
Pups day 4 - pre-cull	[n]	270	274	267	269	272	274	269	273	111	109
- **Viability index**	[%]	99	99	100	100	99	99	99	100	99	99
Pups day 4 - post cull	[n]	184	178	182	183	184	190	184	192	72	78
Pups day 21	[n]	184	178	182	183	184	190	184	192	71	78
- **Lactation index**	[%	100	100	100	100	100	100	100	100	99	100
Sex ratio	[% live males]										
- Day 0		51.5	51.1	35.0	51.5	44.4	50.0	46.3	54.9	49.1	44.5
- Day 21		48.5	48.9	47.0	48.5	55.6	50.0	53.7	45.1	50.9	55.5
Anogenital distance males											
- Day 1	[mm]	3.09	3.15	3.04	**2.95***	2.95	2.94	2.95	**2.72****	nd	nd
- Index d1 [body weight ratio]		0.47	0.47	0.48	0.45	0.47	0.46	0.46	**0.44***	nd	nd
	[%]	100	100	102	96	100	98	98	94		
Anogenital distance females											
- Day 1	[mm]	1.55	1.61	1.55	1.57	1.57	1.58	1.61	1.55	nd	nd
- Index d1 [body weight ratio]		0.25	0.25	0.25	0.25	0.26	0.26	0.26	0.27	nd	nd
Presence areola/nipples males											
- PND 12 dto per litter [mean %		67.0	78.3	57.4	77.5	42.0	48.3	**69.2****	**67.3****	17.8	15.8
Standard Deviation		30.0	30.4	33.9	27.7	33.9	34.2	28.6	36.7	23.8	32.0
- PND 20	[n]	0	0	**1**	**4**	0	0	0	**5**	0	0
- PND 20	[%]	0.0	0.0	**1.0**	**4.2**	0.0	0.0	0.0	**5.0***	0.0	0.0
- PND 20 dto per litter [mean %		0.0	0.0	**1.1**	**2.9**	0.0	0.0	0.0	**5.2***	0.0	0.0
Standard Deviation		0.0	0.0	5.21	13.90	0.0	0.0	0.0	12.72		
Male pup weight	[g]										
- Day 1	[g]	6.6	6.7	6.4	6.5	6.4	6.4	6.5	6.2	6.9	7.0
- Day 4 - pre cull	[g]	10.2	10.1	9.7	9.7	9.9	9.7	9.8	9.3	10.5	10.7
- Day 4 - post cull	[g]	10.3	10.1	9.8	9.7	9.9	9.8	9.8	9.3	10.5	10.7
- Day 7	[g]	16.5	16.3	15.7	15.7	16.2	15.7	15.9	**15.2***	16.9	16.8
- Day 14	[g]	32.8	32.7	32.0	32.0	32.8	31.9	32.5	31.2	35.3	34.3
- Day 21	[g]	52.1	51.4	50.4	**49.6***	51.0	49.5	50.2	**47.8****	56.7	55.4
Male body weight gain											
- Day 4 to 21	[g]	41.9	41.3	40.6	**39.9***	41.1	39.7	40.4	**38.5****	46.1	44.8
	[%]		-1.4	-3.1	-4.8		-3.4	-1.7	-6.3		-2.8
Female pup weight	[g]										
- Day 1	[g]	6.3	6.4	6.2	6.2	6.1	6.2	6.2	5.9	6.6	6.7
- Day 4 - pre cull	[g]	9.9	9.8	9.4	9.4	9.6	9.5	9.5	8.9	10.2	10.1
- Day 4 - post cull	[g]	9.9	9.8	9.5	9.4	9.6	9.5	9.5	9.0	10.3	10.1
- Day 7	[g]	15.9	15.7	15.2	15.2	15.7	15.3	15.4	**14.6***	16.5	16.0
- Day 14	[g]	31.8	31.9	31.3	31.2	31.9	31.1	31.5	30.4	34.3	33.0
- Day 21	[g]	50.2	49.8	48.6	48.1	49.6	48.1	48.9	**46.7****	53.9	52.6
Female body weight gain											
- Day 4 to 21	[g]	40.3	40.0	39.2	38.7	40.0	38.7	39.5	**37.8****	43.7	42.5
	[%]		-0.7	-2.7	-4.0		-3.3	-1.3	**-5.5**		-2.8

[a] Female #362 (F_2, 750ppm) delivering all 10 pups stillborn
[nd] not determined

2. Sex ratio

The sex ratios at day 0 and 21 were not affected by treatment in neither generation. All differences were within the historical control range and not indicative of a treatment-related effect [see Table 5.6.1-6].

3. Pup clinical observations / Anogenital Distance / Presence of Areola and Nipples

For controls one **F_1** pup (#11 of dam #104) anal atresia and a thread like tail was recorded during lactational days 0 – 3 and for one mid-dose pup (#6 of dam #166) lesion of the forelimb from lactation day 1 onwards was recorded. The single occurrence and the lack of a dose-response indicated that the observation in the mid-dose group was not related to treatment. Furthermore, this finding is also occasionally observed in control pups. No clinical observations were recorded in **F_2** pups.

The anogenital distance in high-dose **F_1** male pups was slightly reduced with regard to the absolute distance but did not reach statistical significance when related to the body weight of pups. In high-dose **F_2** male pups the absolute anogenital distance and the body weight ratio were slightly reduced, just reaching statistical significance. Overall the effect on the anogenital distance between **F_1** and **F_2** male pups was comparable, slightly affecting the high-dose group.
There were no effects on the anogenital distance in female pups with regard to absolute distance or the body weight ratio in any generation.

The percentage of animals with present areola/nipples in male **F_1** pups at day 12 after birth was not significantly different between treatment and control groups and did not display a dose response relationship. At day PND 20, areolas/nipples were still present in one mid-dose male pup (#153.3 of dam #153) and four high-dose male pups (#190.1, #190.3, #190.5, #190.6 of dam #190). Only mid-dose pup #153.3 was randomly selected to act as parent (assigned #251) for the **F_2** generation. Clinical observations for **F_1** pup #153.3 showed no effects distinctively different from the group mean with regard to clinical observations and onset of puberty. No clinical observations were recorded for **F_1** pup #153.3 coded as parent #251 during premating and onwards until the day of necropsy. At necropsy, macroscopic (liver lobe torsion) and microscopic (accessory cortical tissue of the adrenal and liver torsion) observation were made and regarded to be without relation to the retention of areola/nipples at PND 20. No significant effects on absolute and relative organ weights neither on sexual maturation, mating, delivery and post-implantation loss were observed. The single incidence of post-implantation loss observed in female #375 paired with male #251 was within the range of the untreated control animals. The remaining implants led to 5 viable male and 5 viable female pups. One animals of each sex was culled PND 4, the remaining pups did not display any clinical effects. Areola/nipples were present in 3 out of 4 (75%) male pups at PND 12 (compared to 70% in the concurrent control group) and 0 out of 4 male pups at PND 20. Male pup #375.3 was raised until day 67 after birth and did not display a difference in sexual maturation compared to the group mean or effects on sperm parameter (1% abnormal sperm vs 2.5 -14% in concurrent control).

The percentage of animals with present areola/nipples in mid- and high-dose **F_2** male pups at day 12 after birth was significantly different from controls without a dose-response relationship. This findings was regarded not treatment related as areola/nipples in untreated control animals of the **F_1** generation were present in about the same number of animals. At PND 20, areolas/nipples were still present in five high-dose **F_2** male pups (#381.2 of dam #381; #385.1 of dam #385; #396.1 and #396.4 of dam #396; #398.3 of dam #398).

A subset of these **F_2** offspring, comprising 5 high-dose males with nipple/ areola retention (#381.2, #385.1, #396.1, #396.4, #398.3) 5 high-dose males without nipple/ areola retention (#386.2, #391.4, #399.2, #384.3, #380.2) and 10 control male animals were further selected to be raised until test week 10.

Clinical observations for **F_2** pups displaying areola/nipple retention had no effects distinctively different from the group mean with regard to clinical observations and onset of puberty [Table 5.6.1-7]. No clinical observations were recorded for **F_2** pups #381.2, #385.1, #396.1, #396.4, #398.3 coded as parents #492, #498, #484, #485, #490, respectively during premating and onwards until the day of necropsy. No significant effects on mating, delivery and post-implantation loss were observed. Areola/nipples were present in 6 out of 39 (15.8%) male pups at PND 12 of the treated F3 pups (compared to 17.8% in the concurrent control group) and 0 out of 39 at PND 20.

Table 5.6.1-7: Anogenital distance / preputial separation of selected male high-dose F_2 pups

Pup generation		F_2					
Dose	[ppm]	1500 ppm					
Pup	[#]	381.2	385.1	396.1	396.4	398.3	Group
Coded as parent	[#]	492	498	484	485	490	Mean
Anogenital distance							
- Postnatal day (PND) 1	[mm]	3.00	2.90	2.50	2.80	2.60	2.94
- Index PND1 [body weight ratio]		0.5	0.47	0.38	0.43	0.37	0.44
	[%]	114	107	86	98	84	100
Preputial Separation							
- Days to criterion		49	46	45	49	47	47.9
- Body weight at criterion	[g]	183.8	192.6	191.2	193.3	195.2	196.8

Overall there was no indication that pups with present areola/nipples at day 20 after birth displayed any adverse or distinctively different effects, on mating, reproduction or developmental or any other parameter assessed with regard to animals of the same treatment group.

4. Body weight

Body weight development was impaired in high dose **F_1** male and female pups [see Table 5.6.1-6]. Mean body weights of the high-dose F_1 male pups and both sexes combined were statistically significantly below the concurrent control values on PND 21 (about 5% and 4% respectively). The body weight change of the high-dose male pups was statistically significantly below the control values during PND 1 - 4 (about 14%) and during PND 14 - 21 and PND 4 - 21 (about 9% and 5%, respectively). The body weight change of the female pups was statistically significantly below the control values during PND 14 - 21 (about 8%). Both sexes combined showed statistically significantly reduced body weight change during PND 1 - 4 and PND 14 - 21 (about 11% and 8%, respectively).

Body weight development was also impaired in high dose **F_2** male and female pups [see Table 5.6.1-6]. Mean body weights of the high-dose (750 ppm) **F_2** male pups, female pups and both sexes combined were statistically significantly below the concurrent control values on PND 7 (about 6 - 7%) and on PND 21 (up to 6%). The body weight change of the high-dose male pups was statistically significantly below the control values during PND 14 - 21 and PND 4 - 21 (about 9% and 6%, respectively). The body weight change of the female pups was statistically significantly below the control values during PND 1 - 4, PND 4 -7, PND 14 - 21 and PND 4 - 21 (about 11%, 8%, 8% and 6% respectively). Both sexes combined showed statistically significantly reduced body weight change during PND 4 – 7, PND 14 - 21 and PND 4 - 21 (about 7%, 9% and 6%, respectively).

No effects on body weights and body weight development were observed in low- and mid-dose **F_1** and **F_2** pups.

5. Organ weights

Mean absolute and relative pup organ weights of the **F_1** pups of all test groups did not show statistically significant differences to the control group

Secondary to the decreased terminal **F_2** pup weights statistically significant changes of absolute brain weight was noted in low and/or high dose pups [see Table 5.6.1-8]. However, relative brain weights were not affected. Thus the changes in absolute brain weights were considered to be spontaneous in nature and not considered toxicologically relevant or adverse.

Table 5.6.1-8: Organ weights of F_2 pups

Generation		F_2 (Males)				F_2 (Males & Females commbined)			
	Dose [ppm]	Absolute weight [g]	Δ%	Relative weight [% of b.w.]	Δ%	Absolute weight [mg]	Δ%	Relative weight [% of b.w.]	Δ%
Terminal weight	0	51.0				50.3			
	300	49.5	(-2.4)			48.8	(-3.0)		
	750	50.2	(-1.6)			49.5	(-1.6)		
	1500	**47.8****	**(-6.3)**			**47.3****	**(-6.0)**		
Brain	0	1.545		3.029		1.519		3.027	
	300	**1.506***	**(-2.5)**	3.073	(1.5)	1.496	(-1.5)	3.099	(2.4)
	750	1.527	(-1.2)	3.058	(1.0)	1.502	(-1.1)	3.018	(-0.3)
	1500	**1.498****	**(-3.0)**	3.134	(3.5)	**1.481****	**(-2.5)**	3.143	(3.8)

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

6. Pup necropsy findings

No treatment-related gross necropsy findings were observed in **F_1**, **F_2** and **F_3** pups.

A few gross necropsy findings were observed in **F_1** and **F_3** pups [see Table 5.6.1-9]. The individual findings were observed in a low incidence, displayed no dose response and were within the historical control range.

Likewise, F_2 pups displayed a low incidence of gross necropsy findings [see Table 5.6.1-9]. All findings were observed at a low incidence and were within the historical control range. The statistically significantly increased number of pups at the mid-dose with post mortem autolysis was considered to be secondary to the increased number of stillbirths in this dose group, an observation considered to be a spontaneous result unrelated to treatment.

All findings were therefore considered to be spontaneous and not related to treatment.

Table 5.6.1-9: Incidence of gross necropsy observations in F_1, F_2 and F_3 pups

Dose [ppm]	0	300	750	1500
	F_1 pups			
Litters evaluated	23	22	23	23
Pups evaluated	226	224	217	220
- Live	224	224	217	219
- Stillborn	2	0	0	1
Anal Atresia	1 (1)	0	0	0
Hemorrhagic Thymus	0	0	1 (1)	0
Diaphragmatic Hernia	0	0	0	1 (1)
Dilated Renal Pelvis	5 (4)	1 (1)	2 (1)	2 (1)
Small Testis	0	0	0	1 (1)
Thread-Like Tail	1 (1)	0	0	0
Total pup necropsy observations	5 (4)	1 (1)	3 (2)	4 (3)
- % affected pups/litter	2.1 ± 4.87	0.3 ± 1.64	1.1 ± 3.75	2.3± 7.35
	F_2 pups			
Litters evaluated	23	24	24	24
Pups evaluated	272	277	284	278
- Live	271	275	**272****	273
- Stillborn	1	2	**12****	5
Post mortem autolysis	0	0	5 (3)	1 (1)
Incisors sloped	0	0	2 (1)	0
Hemorrhagic thymus	1 (1)	0	0	0
Diaphragmatic hernia	0	1 (1)	0	1 (1)
Dilated renal pelvis	2 (1)	0	0	0
Small testes	0	0	0	1 (1)
Small seminal vesicles	0	0	0	1 (1)
Total pup necropsy observations	3 (2)	1 (1)	7 (4)	4 (4)
- % affected pups/litter	1.2 ± 4.1	0.3 ± 1.7	2.1 ± 5.26	1.6 ± 3.70
	F_3 pups			
Litters evaluated	9			10
Pups evaluated	110			110
- Live	110			109
- Stillborn	0			1
Dilated renal pelvis	1 (1)			1 (1)
Dilated Ureter	0			1 (1)
Total pup necropsy observations	1 (1)			2 (2)
- % affected pups/litter	0.9 ± 2.56			2.0 ± 4.29

( ) values in brackets give litter incidence

7. Sexual maturation

Male and female **F_1** or **F_2** pups selected to become **F_1** or **F_2** parental animals were examined for sexual maturation.

Table 5.6.1-10: Sexual maturation of F_1 / F_2 pups

Sex & parameter		Females / Vaginal opening				Males / Preputial separation			
Dose	[ppm]	0	300	750	1500	0	300	750	1500
		F_1 Pups							
Animals per dose		25	25	25	24	25	25	25	24
- Days to criterion		30.0	30.2	**31.1***	**31.4***	40.6	41.4	**44.8****	**48.2****
- Body weight at criterion	[g]	89.4	87.1	91.5	90.2	169.5	170.2	**185.7****	**198.4****
		F_2 Pups							
Animals per dose		25	25	25	25	25	25	25	25
- Days to criterion		30.1	31.2	30.6	**32.1****	40.1	**41.8****	**45.1****	**47.9****
- Body weight at criterion	[g]	88.8	91.5	90.3	92.9	168.4	168.7	**191.1****	**196.8****

* $p \leq 0.05$, ** $p \leq 0.01$ (Dunnett-test, two-sided)

Vaginal opening was slightly but significantly delayed in mid-dose **F_1** as well as high-dose **F_1** and **F_2** females [see Table 5.6.1-10]. This apparent delay was considered secondary to the lower body weight during the major phase of sexual maturation. Furthermore, these values reflect the normal range of biological variation inherent in the strain of rats used for this study. In fact, the mean age of vaginal opening was at the lower limit of the historical control range in control and low-dose females, whereas the mid- and high-dose values are well within the range of the historical control data of the test facility (range: 30.0 – 32.4 days, mean 31.3 days). This holds true also for the body weights at the time of vaginal opening was observed (historical control range: 86.4 to 99.6 g).

In low dose **F_2** males as well as in mid- and high-dose **F_1** and **F_2** males, preputial separation was statistically significantly delayed. The apparent delay in low-dose males did not correspond to an increase in body weight at criterion when compared to controls, suggesting that the delay in pubertal onset was secondary to a generally slower development, rather than a primary result of a specific mode of toxicity. In addition, the delay in puberty of low-dose males was well within the historical control values while the concurrent controls were close to the minimum and well below the mean of the historical control range (range: 39.7 – 42.5 days, mean 41.7 days).

In the mid and high-dose **F_1** and **F_2** males the statistically significant rise in the age at preputial separation corresponded to an increase in body weight (historical control range for body weight: 156.5 – 180.5). Thus, while slower body weight development may have played a role in the delay in sexual development, the principle cause of this effect was an additional, more specific mode of action. Since the delay was also outside the historical control range, it was therefore considered to be adverse. However, the delay in preputial separation did not impact on mating or reproduction of next generation animals and can thus be considered a transient effect not leading to reproductive toxicity.

H. CLINICAL CHEMISTRY

Clinical chemistry investigation in **F_0** parents revealed a statistically significant increase in cholesterol in males at the low- and high dose level. In females alanine amino transferase (AL(A)T) activity as well as total bilirubin and triglycerides at low- and mid-dose were significantly higher than in control animals.

In mid- and high-dose **F_1** parental groups of both sexes as well as in low-dose females creatinine levels were significantly increased. In mid-dose **F_1** males glucose levels were below and in low-dose males cholesterol levels were significantly higher as in controls. In mid- and high-dose **F_1** females alanine aminotransferase (AL(A)T) activities were decreased.

Table 5.6.1-11: Clinical chemistry findings of rat administered BAS 610 F throughout 2 generations

Sex		Males				Females			
Dose	[ppm]	0	300	750	1500	0	300	750	1500
		F_0 generation							
AL(A)T	[µkat/l]	0.84	0.87	0.94	1.11	0.62	**0.73***	**0.70***	0.63
Creatinine	[mmol/l]	56.6	55.6	55.9	60.3	54.6	57.0	56.4	55.9
Glucose	[mmol/l]	7.27	7.01	7.19	6.74	5.81	5.70	5.38	5.41
Total Bilirubin	[µmol/l]	2.38	2.69	2.44	2.51	2.63	**3.37***	**3.36****	2.86
Cholesterol	[mmol/l]	1.86	**2.19****	1.98	**2.12***	1.63	1.94	1.81	1.69
Triglycerides	[mmol/l]	0.73	1.00	0.85	0.85	0.49	**0.72***	**0.69****	0.60
		F_1 generation							
AL(A)T	[µkat/l]	0.86	0.83	0.80	0.80	0.65	0.67	**0.58****	**0.56****
Creatinine	[mmol/l]	54.5	55.0	**57.4***	**58.5****	52.3	**55.4***	**55.8***	**57.2****
Glucose	[mmol/l]	7.09	6.84	**6.30****	6.65	5.35	5.08	5.21	4.93
Total Bilirubin	[µmol/l]	2.48	2.56	2.47	2.39	3.33	3.34	3.48	3.74
Cholesterol	[mmol/l]	1.90	**2.24***	1.92	1.78	1.69	1.80	1.66	1.69
Triglycerides	[mmol/l]	1.38	1.12	1.09	1.30	0.79	1.02	0.82	1.11

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

The significant increase in cholesterol in parental **F_0** animals was well within the historical control range (range: 1.51 – 2.23 mmol/L) whereas the increased alanine amino transferase (AL(A)T) activity as well as total bilirubin and triglycerides levels in females did not display dose dependency [see Table 5.6.1-11**].** Therefore, all mentioned parameter alterations in parental **F_0** animals were regarded as incidental and not treatment-related.

The significantly increased creatinine levels in **F_1** parental animals occurred above the historical control range in males (range males: 48.3 – 55.9 mmol/L) and within the range in females (range females: 52.2 -62.7 mmol/L). No other changes were noted in the affected animals. Therefore these alterations were regarded as treatment-related but not adverse (ECETOC, Technical Report No. 85, 2002). The effects on male **F_1** glucose levels as well as cholesterol levels occurred without displaying a dose-response relationship. Decreased alanine aminotransferase (AL(A)T) activities in **F_2** females were within the historical control range (range: 0.42-0.81 µkat/L). Therefore these mentioned alterations in parental **F_1** animals were regarded as incidental and not treatment-related.

I. Parental terminal investiations

1. Organ weights

Organ weight determination in parental animals revealed a number of significant changes of absolute and/or relative organ weights [Table 5.6.1-12 for males and Table 5.6.1-13 females].

The increases of absolute and/or relative adrenal weights in **F_0** and **F_1** parental rats was accompanied by histopathological findings and were regarded to be treatment-related but non-adverse (see discussion in histopathology section).

Table 5.6.1-12: Organ weights of F_0 and F_1 male parental animals

Generation		F_0 Males				F_1 Males			
	Dose [ppm]	Absolute weight	%[&]	Relative weight [% of b.w.]	%[&]	Absolute weight [mg]	%[&]	Relative weight [% of b.w.]	%[&]
Terminal weight [g]	0	403.46				404.716			
	300	403.356	(0.0)			407.996	(0.8)		
	750	393.324	(-2.5)			404.804	(0.0)		
	1500	394.6	(-2.2)			393.860	(-2.7)		
Adrenal gland [mg]	0	60.68		0.015		63.52		0.016	
	300	58.208	(-4.1)	0.014	(-6.7)	62.16	(-2.1)	0.015	(-6.3)
	750	62.52	(3.0)	0.016	(6.7)	65.80	(3.6)	0.016	(0.0)
	1500	**67.88***	**(11.9)**	**0.017****	**(13.3)**	**70.00***	**(10.2)**	**0.018***	**(13.2)**
Brain [g]	0	2.069		0.516		2.078		0.516	
	300	2.044	(-1.2)	0.510	(-1.1)	2.107	(1.4)	0.520	(0.8)
	750	2.033	(-1.7)	0.520	(0.8)	2.074	(-0.2)	0.517	(0.2)
	1500	2.048	(-1.0)	0.522	(1.2)	**2.035***	**(-2.1)**	0.520	(0.8)
Cauda epididymis [g]	0	0.437		0.109		0.473		0.117	
	300	0.406	(-7.1)	0.101	(-7.3)	**0.447***	**(-5.5)**	0.110	(-6.0)
	750	0.400	(-8.5)	0.103	(-5.5)	**0.424****	**(-10.4)**	**0.105****	**(-10.3)**
	1500	0.396	(-9.4)	0.101	(-7.3)	0.441	(-6.8)	0.113	(-3.4)
Epididymides [g]	0	1.140		0.284		1.197		0.297	
	300	1.071	(-6.1)	0.267	(-6.0)	1.164	(-2.8)	0.287	(-3.4)
	750	1.080	(-5.3)	0.276	(-2.8)	**1.131****	**(-5.5)**	0.281	(-5.4)
	1500	1.093	(-4.1)	0.279	(-1.8)	1.156	(-3.4)	0.295	(-0.7)
Kidneys [g]	0	2.566		0.637		2.488		0.616	
	300	2.454	(-4.4)	0.611	(-4.1)	2.405	(-3.3)	0.591	(-4.1)
	750	2.388	(-6.9)	0.608	(-4.6)	2.383	(-4.2)	0.590	(-4.2)
	1500	2.470	(-3.7)	0.628	(-1.4)	2.437	(-2.1)	0.620	(0.7)
Pituitary gland [mg]	0	10.40		0.003		12.16		0.003	
	300	10.44	(0.4)	0.003	(0.4)	12.48	(2.6)	0.003	(1.8)
	750	10.68	(2.7)	0.003	(5.3)	11.76	(-3.3)	0.003	(-3.3)
	1500	10.40	(0.0)	0.003	(2.3)	12.24	(0.7)	0.003	(3.4)
Seminal vesicle [g]	0	1.312		0.328		1.330		0.330	
	300	1.210	(-7.8)	0.301	(-8.2)	1.268	(-4.7)	0.312	(-5.5)
	750	**1.148****	**(-12.5)**	**0.293****	**(-10.7)**	1.262	(-5.1)	0.314	(-4.8)
	1500	1.187	(-9.5)	0.303	(-7.6)	1.233	(-7.3)	0.315	(-4.5)
Thyroid glands [mg]	0	20.80		0.005		24.56		0.006	
	300	23.36	(12.3)	**0.006***	**(12.3)**	26.00	(5.9)	0.006	(5.0)
	750	22.04	(6.0)	0.006	(8.7)	26.64	(8.5)	0.007	(8.5)
	1500	22.72	(9.2)	**0.006****	**(11.7)**	26.92	(9.6)	0.007	(12.6)

* $p \leq 0.05$, ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test (two-sided))

[&] Values may not calculate exactly due to rounding of figures. The values given are based on the unrounded means

Table 5.6.1-13: Organ weights of F_0 and F_1 parental females

Generation		F_0 Females				F_1 Females			
	Dose [ppm]	Absolute weight	%[&]	Relative weight [% of b.w.]	%[&]	Absolute weight [mg]	%[&]	Relative weight [% of b.w.]	%[&]
Terminal weight [g]	0	231.024				235.136			
	300	235.196	(1.8)			232.824	(-1.0)		
	750	229.792	(-0.5)			236.116	(0.4)		
	1500	**223.396***	**(-3.3)**			227.364	(-3.3)		
Adrenal gland [mg]	0	76.12		0.033		73.40		0.031	
	300	70.36	(-7.6)	**0.030***	**(-9.1)**	69.56	(-5.2)	0.030	(-3.2)
	750	73.00	(-4.1)	0.032	(-3.0)	73.56	(0.2)	0.031	(0.0)
	1500	79.08	(3.9)	0.035	(6.1)	**83.80****	**(14.2)**	**0.037****	**(19.4)**
Brain [g]	0	1.928		0.836		1.933		0.825	
	300	**1.973***	**(2.3)**	0.841	(0.6)	1.962	(1.5)	0.845	(2.4)
	750	1.949	(1.1)	0.851	(1.8)	1.900	(-1.7)	0.806	(-2.3)
	1500	1.932	(0.2)	0.867	(3.7)	1.912	(-1.1)	0.844	(2.3)
Kidneys [g]	0	1.756		0.761		1.666		0.709	
	300	1.722	(-1.9)	0.732	(-3.8)	1.587	(-4.7)	0.683	(-3.7)
	750	1.716	(-2.3)	0.748	(-1.7)	1.590	(-4.6)	**0.673***	**(-5.1)**
	1500	**1.652****	**(-5.9)**	0.740	(-2.8)	**1.507****	**(-9.5)**	**0.663****	**(-6.5)**
Liver [g]	0	6.042		2.617		6.278		2.672	
	300	6.107	(1.1)	2.596	(-0.8)	6.136	(-2.3)	2.634	(-1.4)
	750	6.091	(0.8)	2.652	(1.3)	6.414	(2.2)	2.718	(1.7)
	1500	6.196	(2.6)	**2.773***	**(5.7)**	6.331	(0.8)	**2.787***	**(4.3)**
Pituitary gland [mg]	0	13.08		0.006		14.36		0.006	
	300	13.08	(0.0)	0.006	(-1.7)	**12.60****	**(-12.3)**	**0.005***	**(-11.4)**
	750	13.04	(-0.3)	0.006	(0.2)	**12.72***	**(-11.4)**	**0.005***	**(-11.8)**
	1500	13.00	(-0.6)	0.006	(2.7)	**12.52****	**(-12.8)**	**0.005***	**(-9.8)**

* $p \leq 0.05$, ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test (two-sided))

[&] Values may not calculate exactly due to rounding of figures. The values given are based on the unrounded means

Most of the statistically significant absolute and relative organ weight changes were considered to be secondary to the decreased terminal body weights. In these cases no histopathological correlate was observed, which indicated a cause for the organ weight change. Thus these organ weight changes were considered unrelated to treatment.

In the following all organ weight changes are discussed to which the above does not apply:

- The change in absolute brain weight was not dose-dependent (**F_0** females) and/or relative brain weight was not affected (**F_0** females, **F_1** males) (no histopathology performed).
- The decrease in absolute cauda epididymis weight in low- and mid-dose **F_1** males was not dose-dependent and/or did not affect relative cauda epididymis weights. (no histopathology performed).
- The decrease of absolute kidney weights in high dose **F_0** and **F_1** females were within the range of historical control data (mean: 1.674 g; min. 1.391 g – max. 1.866 g) and the relative kidney weights were only slightly below the range of historical control data (min. 0.676% - max. 0.781%). (no histopathology performed).
- The increase of relative thyroid weights in low- and high dose **F_0** males was not dose-related and not seen in males of the following generation (no histopathology performed).
- The increased relative liver weight in high-dose **F_0** (+6%) and **F_1** (+4%) females was related to the (significantly in **F_0**) decreased terminal body weights (-3% in both parental generations) (no histopathology performed).
- The pituitary weights in parental **F_1** animals were clearly within the range of historical control data (mean: 12.536 mg; min. 10.680 mg – max. 14.560 mg), whereas the mean pituitary weight of control females (14.36 mg) was in the upper range.

Therefore, all above listed organ weight changes were not considered to be treatment-related.

2. Macroscopic lesions

All gross lesions either occurred singularly or with a higher incidence in control animals. Therefore, all of them were considered to be spontaneous lesions in origin and were not related to treatment

3. Histopathology

Treatment-related histopathological changes were observed in the adrenals. Incidences and average severity gradings for **F_0** and **F_1** parental animals are given in Table 5.6.1-14.

An increased incidence of cytoplasmic vacuolation especially in *zonae fasciculata* and *glomerulosa* was observed in the adrenal cortex of high dose parental **F_0** animals. In high-dose males the cytoplasmic vacuolation was characterized either by multiple, round, clearly demarcated to coalescing vacuoles of varying size or by more microvesicular vacuoles whereas in all affected females the vacuolation pattern was only of the microvesicular type, giving the cytoplasm of adrenal cortex cells a foamy appearance. This also applies to the slight vacuolation observed in control animals of both sexes.

The increased incidence of cytoplasmic vacuolation was also observed in **F_1** animals, affecting both sexes at mid- and high-dose treatment level. As in the previous generation, males where affected by cytoplasmic vacuolation which was characterized either by multiple, round, clearly demarcated to coalescing vacuoles of varying size or by more microvesicular vacuoles and females by a vacuolation pattern which was only of the microvesicular type. The latter observation was also seen in control animals.

The increase of vacuolation was correlated on an individual animal basis with increased adrenal weights in high-dose **F_1** males (partly) and females but not to adrenal weights in **F_0** animals.

Because the severity of the vacuolation was in most affected animals only minimal or slight and because no other histopathological findings as cellular degeneration were observed, the increased occurrence of vacuolation was considered to be non-adverse.

Table 5.6.1-14: Incidence of selected histopathological lesions in F_0 and F_1 parental rats

Sex		Males				Females			
Dose [ppm]		0	300	750	1500	0	300	750	1500
Animals in group		25	25	25	25	25	25	25	25
		F_0 - Generation							
Adrenals	# examined	25	25	25	25	25	25	25	25
- Vacuolation increased		2	1	4	**17****	1	2	2	**13****
		[1.0]	*[1.0]*	*[1.5]*	*[1.6]*	*[1.0]*	*[1.0]*	*[1.0]*	*[1.3]*
		F_1 – Generation							
Adrenals	# examined	25	25	25	25	25	25	25	25
- Vacuolation increased		2	3	6	**16****	1	1	**10****	**23****
		[1.0]	*[1.0]*	*[1.2]*	*[2.0]*	*[2.0]*	*[1.0]*	*[1.0]*	*[1.9]*

$ [ ] mean severity grading; histopathological findings were graded very slight/minimal (Grade 1), slight (Grade 2), moderate (Grade 3), severe/marked (Grade 4) and very severe/massive/extreme (Grade 5). The mean severity is the sum of the gradings divided by the incidence

* $p < 0.05$; ** $p < 0.01$; (Pairwise Fisher's test)

All other findings noted were either single observations, or were similarly in distribution pattern and severity in control rats compared to treatment groups. All of them are considered to be incidental and spontaneous in origin and without any relation to treatment

4. Differential ovarian follicle count

The results of the differential ovarian follicle count (DOFC) – comprising the numbers of primordial and growing follicles, as well as the combined incidence of primordial plus growing follicles – did not reveal significant deviations between controls and the high dose group [see Table 5.6.1-15].

Table 5.6.1-15: Ovarian follicle count in F_1 maternal females

	Absolute number			Mean number		
Group	Primordial	Growing	Primordial + growing	Primordial	Growing	Primordial + growing
Control	4329	516	4845	173.16	20.64	193.80
1500 ppm	4108	514	4622	164.32	20.56	184.88

III. CONCLUSIONS

Under the conditions of the present 2-generation reproduction toxicity study the **NOAEL** for **general, systemic toxicity** is 750 ppm (83 mg/kg bw/d) for the $\mathbf{F_0}$ and $\mathbf{F_1}$ parental rats, based on decreased food consumption and body weight/body weight gain during gestation, observed at the LOAEL of 1500 ppm (156 mg/kg bw/d).
The **NOAEL** for **fertility** and **reproductive performance** for the $\mathbf{F_0}$ and $\mathbf{F_1}$ parental rats is at least 1500 ppm (156 mg/kg bw/d).
The NOAEL for **developmental toxicity** in the **female** $\mathbf{F_1}$ and $\mathbf{F_2}$ progeny is 750 ppm (85 mg/kg bw/d), based on reduced pre-weaning pup body weights at 1500 ppm. In **males**, the NOAEL for developmental toxicity is 300 ppm (32 mg/kg bw/d) due to the delay in the onset of puberty that occurred at 750 ppm.

BASF assessment of the 2-generation reproductive toxicity study BASF DocID 2013/1251918: Treatment of Wistar rats for 2-generations with BAS 610 F (iprodione) at dose levels of 0 (control), 300 ppm (33mg/kg bw/d), 750 ppm (83 mg/kg bw/d) and 1500 ppm (156 mg/kg bw/d) caused parental toxicity at the highest dose tested, based on decreased food consumption and body weight/body weight gain in females during gestation. No effects were apparent with regard to clinical pathology in either generation. A marginal but statistically significant increase in mean percent abnormal sperm was observed from low-dose on. Thereby, most of the abnormalities comprised breakage of the sperms in heads and tails as well as smaller heads. The statistically significant increase in abnormal sperm in the F_1 generation was assessed on an individual animal basis to be within the concurrent control range (0-14%) and 97.5th percentile historical control range (0-11%) with the exception of one mid dose male, which was within the 100th percentile historical control range (0 – 22%), and two high dose male animals, of which one was at the border of the concurrent control (14.5%) and one above the entire historical control range (24%). Neither fertility was affected nor was there any histopathological change in testis or epididymides. Therefore the findings were regarded as incidental and not treatment related. Regarding pathology, the target organ was the adrenal gland in parental animals, which was affected treatment related but non-adverse in organ weight (up to 13.3 and 19.4% relative weight increase in males and females, respectively) and histopathologically by cytoplasmic vacuolation. There were no indications from clinical examinations as well as gross and histopathology, that iprodione adversely affected the fertility or reproductive performance of the **F_0** or **F_1** parental animals up to and including the highest administered dose. Estrous cycle data, mating behavior, conception, gestation, parturition, lactation and weaning parameters as well as sexual organ weights and gross and histopathological findings (including differential ovarian follicle counts in the **F_1** females) did not indicate any substance-related effects.
For all liveborn female pups of the **F_0** and **F_1** parents, no test substance-induced signs of developmental toxicity were noted at dose levels as high as 750 ppm. Postnatal survival as well as post-weaning development of the offspring of these test groups until sexual maturity remained unaffected by the test substance. Furthermore, clinical and/or gross necropsy examinations of the **F_1** and **F_2** female pups revealed no adverse findings. The same applies to liveborn male pups of the **F_0** and **F_1** parents at dose levels as high as 750 ppm, except for a test-substance related delay in onset of puberty. At 1500 ppm, pup body weights were reduced towards weaning in the **F_1** pups; high-dose **F_2** pup weights were reduced by mid-lactation. The anogenital distance was statistically significantly reduced at the highest dose tested in **F_1** and **F_2** male pups. A corresponding statistically significant reduction in anogenital index was only noted in the males of the **F_2** generation, which was however of comparable magnitude to **F_1** pups. Therefore, effects on anogential distance were regarded as incidental and not treatment related. Presence of areola/nipple at PND 20 was recorded in male animals of the high dose-group at weaning in both generations (**F_1**: 4 affected pups from 1 litter; **F_2**: 5 affected pups from 5 litters). One **F_1** male offspring with areola/nipple at weaning was also noted in the mid-dose group. Affected **F_2** males when paired with untreated females produced healthy **F_3** offspring with no evidence for any nipple/areola presence at PND 20. Moreover, follow up of **F_1** and **F_2** pups with present areola/nipple at PND 20 at weaning did not indicate any adverse or distinctively different effects, on mating, reproduction or developmental or any other parameter assessed with regard to animals of the same treatment group.

The onset of female puberty was statistically significantly delayed in **F_1** mid- and high-dose animals as well as high-dose **F_2** animals. The delayed onset in female puberty was not considered to be treatment related, as the statistical significance is mainly due to unusually low concurrent control group values which are at the lower limit of the historical control range. Moreover, the day of puberty onset as well as body weight at criterion, were well within the historical control ranges. The delayed onset in male puberty at 1500 ppm and 750 ppm was outside the historical control range, whereas the effect in low-dose males was well within the historical control range. Mean body weight at criterion of **F_2** low-dose males was almost identical to the concurrent control value. The delayed puberty onset was associated with reduced body weight gain at 1500 ppm but not at 750 ppm, overall indicating a specific effect of iprodione on male sexual maturation. The delay in onset of male puberty did not increase in severity form the first to the second generation it did not impact on mating or reproductive performance of next generation animals nor were any additional effects observed on reproductive organs. Overall sexual development was delayed in male animals at ≥ 750 ppm (82 mg/kg bw), which was of comparable severity in both generations. As no further reproductive parameter were affected in this study, this transient effect is considered to be of low overall severity.

Information from standard regulatory and mechanistic studies for iprodione indicate a general inhibition of steroidogenesis which is the most likely causative effect for the effects on delay in sexual development. These studies consistently identify NOAEL values at dose levels of approximately 30 mg/kg bw/d or greater for endocrine related effects, which coincides with the observed NOAEL of the effects on sexual development in the new 2-generation study. Consistent LOELs for endocrine related effects were reported to occur at around 70 mg/kg bw/d, which again coincides roughly with the LOAEL of 82 mg/kg bw/d for the effects on male sexual development detected in this study.

CA 5.6.2 Developmental toxicity studies

(Studies presented in the original Annex II Dossier)

Rat

Coquet, 1973a (BASF DocID C022944): Study of the teratogenic activity of the product 26019 RP by oral route in the OFA rat.

Groups of female OFA (Sprague Dawley) rats (25-30 animals/group) were given oral doses of 0 (control) – 100, 200 or 400 mg/kg bw/day between Day 5 and 15 of gestation.
There was a slight decrease in body weight gain in the females in the high dose group. The conception rate and mean number of implantations per fertile female in the high dose group were reduced compared to control values. The results of detailed fetal examinations showed no indication of an embryotoxic or teratogenic effect of iprodione.

Conclusion:
The No-Observed-Effect-Level (NOEL) in this study was 200 mg/kg bw/day of iprodione.

Tesh, et al. 1986 (BASF DocID C023030): iprodione (technical grade): Teratology study in the rat.

The embryo/fetal toxicity and teratogenicity of iprodione (technical grade: 94.2 %) were evaluated in sexually mature female Sprague Dawley rats (CD strain). Test material was administered by gastric intubation on gestation days 6-15 inclusive at dosages of 40, 90 or 200 mg/kg bw/day. Control animals received the vehicle, 0.5% w/v aqueous methyl cellulose mucilage throughout the same period.
No clinical signs of toxicity, adverse effects on maternal bodyweight or food intake, or maternal deaths were observed during the study. No significant differences were found between control and treated groups with regard to fetal weights and sex ratios, mean number of viable fetuses, total resorptions, corpora lutea, and implantations. Further, no fetal malformations or anomalies were associated with any of the test material dosages. The slightly lower fetal bodyweight and increased incidence of space between the body wall and organs in the high dose group were considered to be an indication of delayed fetal development. However, neither parameter was greatly different from controls and both were well within the laboratory's range of historical control values.

Conclusion:
iprodione showed no teratogenic property at any dose level in the rat; delayed fetal development was observed at 200 mg/kg/day. Although no maternal toxicity was observed in this study at 200 mg/kg bw/day, signs of maternal toxicity were apparently observed at 120, 240 mg/kg bw/day and above in a preliminary study (increase in the average number of late resorptions per litter at 240 mg/kg bw/day with total litter resorptions at 400 mg/kg bw/day). Therefore, the NOEL for embryofetal and maternal toxicity was considered to be 90 mg/kg bw/day.

Repetto-Larsay, 1997 (BASF DocID C022658): iprodione: Toxicology study in pregnant rat by gavage to examine sex differentiation.

Sperm-positive CD rats were exposed to technical iprodione (batch 9426801; purity: 971 g/kg) by gavage from Days 6 to 19 of gestation. The doses given were 20, 120 or 250 mg/kg bw/day in suspension in aqueous solution of 0.4 % methylcellulose 400. The sperm-positive females were allocated to groups (25 females per group); the spermpositive day being Day 0 of gestation. The volume of administration was 10 mL/kg based on the most recent body weight recorded. A positive control group of 25 females was exposed to 50 mg/kg/day of flutamide and processed as the other study groups. Maternal body weights were recorded for all the females on Days 0, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20. Food consumption was also measured for all the females during the interval Day 0-Day 6, daily from Days 6 to 20. Clinical observations were recorded daily. At scheduled sacrifice, on gestation Day 20, the gravid uterine weight was recorded and the dams were evaluated for number of corpora lutea, number and status of implantations (resorptions, dead and live foetuses). Live foetuses were removed from the uteri, counted, weighed, sexed and examined externally. Placental weights and anogenital distances of live foetuses were also recorded.
Under the conditions of this study, treatment of pregnant rats from gestation Days 6 to 19 with iprodione at doses ranging from 20 to 250 mg/kg bw/day caused a severe maternal toxic effect at 250 mg/kg bw/day producing mortality in three females and necessitating the premature termination of six animals. Maternal toxicity was also observed at 120 mg/kg bw/day since there were macroscopic changes in the adrenals and a statistically significant reduction in body weight change, particularly at the end of the treatment period (Day 16 to 20). Corrected body weight change was decreased but no change in gravid uterine weight was recorded which constitutes a further confirmation of maternal toxicity. No apparent maternal effect was observed in animals treated at 20 mg/kg bw/day. No morphologically abnormal differentiation in external and internal genitalia was found in any iprodione exposed foetus. The statistically significant reduction in absolute anogenital distance observed at 120 or 250 mg/kg bw/day in male foetuses was not considered biologically relevant since these differences observed when compared to control values were slight to minimal and since these dose levels induced maternal toxicity at a critical period of development (Days 16-20). In addition, when the anogenital index was calculated, no differences were observed at any dose level when compared to the controls. No foetal effects were observed with iprodione at 20 mg/kg bw/day.

Conclusion
This study demonstrates that iprodione has no effect on sex differentiation and no specific effect on the anogenital distance of male foetuses when administered to the pregnant females during all period of organogenesis.

Rabbit

Coquet, 1973b (BASF DocID C022989): Study of the teratogenic activity of the product 26019 RP by oral route in the rabbit.

Female New Zealand rabbits (15-17 animals/group) were given oral doses of 0, 100, 200 or 400 mg of iprodione (100 % a.i.)/kg bw/day between Day 6 and 16 of gestation.
Mortality was observed in the high dose group (9 of 17 females died during the treatment period). Body weight gain, over the treatment period, was slightly reduced at 100 mg/kg bw/day and a dose-related weight loss occurred at 200 mg/kg bw/day and above. The middle dose produced total litter resorption in 3 of the 13 gestating females. The other 10 females in this group produced 67 viable and normal fetuses and 1 fetus with multiple malformations. Fetal weight was also reduced at 200 mg/kg bw/day and above. All fetuses in the control and low dose groups were normal and viable. Detailed examination of the fetal organs and skeletons showed that iprodione was not teratogenic at any of the three dose levels tested.

Rodwell, 1985 (BASF DocID C032281): A teratology study in rabbits with iprodione

The embryo/fetal toxicity and teratogenicity of iprodione (technical grade: 95.0 %) were assessed in New Zealand White Rabbits. Test material was administered by gavage to artificially inseminated animals on gestation days 6-18. Dose levels were 0, 20, 60 and 200 mg/kg bw/day (18 females/group). Surviving rabbits were sacrificed on gestation Day 29 and cesarean sections were performed.
No treatment related mortality was observed in this study. Marked signs of maternal toxicity were observed in the 200 mg/kg bw/day group with animals showing significant loss in body weights and decreased food consumption during the treatment period. Seven abortions were observed in the 200 mg/kg bw/day group and these were considered to be a secondary effect of maternal toxicity. Two females treated at 200 mg/kg bw/day also showed total litter resorption resulting in a slight increase in post-implantation loss. Animals in the 60 mg/kg bw/day group showed a body weight loss during the first week of iprodione treatment followed by a slight decrease in body weight gain during the latter portion of the treatment period. Although this weight loss was not statistically significant, it could indicate slight maternal toxicity. No evidence of toxicity was noted in either the 0 or 20 mg/kg bw/day groups. No statistical differences were found between control and treated groups with regard to fetal weights and sex ratios, mean number of viable fetuses, total resorptions, implantation sites, and corpora lutea. No treatment-related fetal malformations or developmental variations were observed in any of the groups.

In summary
iprodione showed no evidence of teratogenic property at any dose level in the rabbit. The NOEL for embryo-fetal toxicity is established at 60 mg/kg bw/day based on increased abortions and post-implantation loss at 200 mg/kg bw/day. The NOEL for maternal toxicity is established at 20 mg/kg bw/day, based on the slight effect on maternal body weight observed at 60 mg/kg bw/day. Therefore, the NOEL for all effects in this study is established at 20 mg/kg bw/day.

CA 5.7 Neurotoxicity Studies

Studies presented in the original Annex II Dossier (1995) or the update of the Annex II Dossier (1997): Iprodione does not have any chemical function known to be associated with neurotoxicity. Neither acute nor subchronic delayed neurotoxicity was considered to be done due to the fact that no neurotoxic symptom had ever been observed in any toxicity study on any animal conducted with iprodione.

Submission of not yet peer-reviewed studies in this AIR3-Dossier: No new neurotoxicity studies have been performed.

CA 5.7.1 Neurotoxicity studies in rodents

Not required

CA 5.7.2 Delayed polyneuropathy studies

Not required

CA 5.8 Other Toxicological Studies

CA 5.8.1 Toxicity studies of metabolites

Iprodione is extensively metabolized in all matrices (mammal, plant, and soil) resulting in numerous metabolites identified. With regard to toxicological relevance the metabolites presented in Table 5.8.1-1 have to be taken into consideration and are addressed here.

Studies on metabolites presented in the original Annex II Dossier (1995) or the update of the Annex II Dossier (1997):

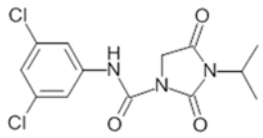
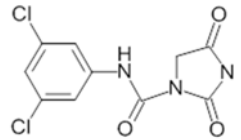
No studies on relevant metabolites have been part of the original Annex II Dossier.

Submission of not yet peer-reviewed studies in this AIR3-Dossier:

A relevance assessment has been performed according to the TTC (Threshold of Toxicological Concern) concept, which is the approach as described in the 'Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment' (EFSA Journal 2012; 10(07): 2799). It is laid down in this EFSA Scientific Opinion that 'the TTC approach is the most appropriate tool to evaluate the toxicological relevance of metabolites associated with chronic dietary exposure. The TTC values, established for genotoxic and toxic compounds based on the Cramer et al., (1978; Food Cosmet. Toxicol. 16, 255–276) scheme, were considered sufficiently conservative'. Recently, the original data base of the TTC concept was expanded with chemicals where reproductive and developmental data is available (Laufersweiler, MC et al., *Regulatory Toxicology and Pharmacology 62 (2012) 160–182*). The assessment indicated that, 'for each Cramer Class, the reproductive and developmental endpoints would be protective at the corresponding general TTC tiers derived in the original data base', therefore increasing confidence that the TTC concept is sufficiently conservative. In accordance to the scientific opinion and agreed with the RMS, metabolites where placed into common toxicological relevance assessment groups, based on structural similarity and metabolic pathway concordance. Metabolite grouping is indicated in Table 5.8.1-1. Quantitative exposure to metabolites in rat metabolism studies was also considered in the relevance assessment.

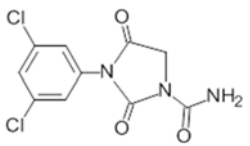
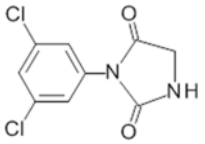
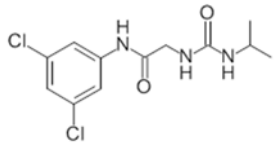
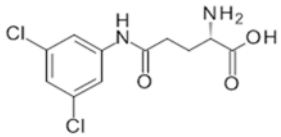
For all metabolites identified with potential relevance or as corresponding group members presence for potential structural alerts was evaluated with different SAR/QSAR models. Models used, were OASIS TIMES and VEGA. However, the QSAR predictions obtained are limited in reliability as most of the structures evaluated were not in the prediction domain. Thus, given the structural relationship of the metabolites evaluated inter alia and in relation to the parent molecule iprodione, the predicted alerts were compared to those for the parent and those metabolites where toxicological data were available in order to overcome the limitations of the predictions made.

Table 5.8.1-1: Metabolites of iprodione considered for potential toxicological relevance

Trivial Name Reg.No.	Group	Structure	Study	Species (Strain)/Test system	Findings	References (BASF DocID)
RP30228 5079647 Page 134	1A		Acute oral toxicity Acute dermal Local irritation Microbial Mutagenicity Assay (Ames) In vitro Micronucleus In vivo Micronucleus In vitro AR binding 13 weeks oral feeding	CD-1 mice CD rats CD rats NZW rabbits S. typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 Chinese hamster V79 cells NMRI mice Human mammary gland cancer cell SD rats	LD50 > 10,000 mg/kg bw/d LD50 > 2,500 mg/kg bw/d LD50 > 2,500 mg/kg bw/d No eye nor skin irritation Negative +/- metabolic act. Positive +/- metabolic act. Negative No AR binding NOAEL 234 - 252 mg/kg bw/d	C022990 C043629 2013/1247542 2013/1286156 R014678 C043621
RP36112 5079623 Page 176			In vitro mechanistic In vitro AR binding	Porcine Leydig Cell Human mammary gland cancer cell	May inhibit T secretion No AR binding	C029154 R014678

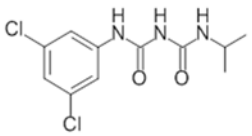
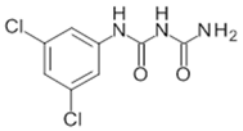
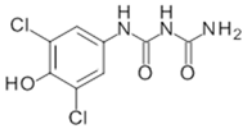
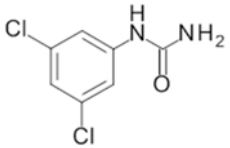
T = Testosterone
act. = activation

Table 5.8.1-1 (continued)

Trivial Name Reg.No.	Group	Structure	Study	Species (Strain)/Test system	Findings	References (BASF DocID)
RP32490 5079628 Page 178	**1B**		**In vitro mechanistic** **In vitro AR binding**	**Porcine Leydig Cell** **Human mammary gland cancer cell**	**Does not inhibit T secretion** **No AR binding**	**C029154** **R014678**
RP25040 207099 Page 179			**Acute oral toxicity** **Local irritation** **Microbial Mutagenicity Assay (Ames)** **In vitro mechanistic** **In vitro AR binding**	**CD-1 mice** **NZW rabbits** **S. typhimurium strains TA98, TA100, TA1535 and TA1537** **Porcine Leydig Cell** **Human mammary gland cancer cell**	**LD50 = 1125 mg/kg bw/d** **No skin irritation** **Negative +/- metabolic act.** **Does not inhibit T secretion** **Very weak AR binding**	**R014584** **C043657** **C043625** **C029154** **R014678**
RP37176 5079612 Page 193	**2A**		**Acute oral toxicity** **Microbial Mutagenicity Assay (Ames)** **In vitro Micronucleus**	**CD-1 mice** **S. typhimurium strains TA98, TA100, TA1535 and TA1537** **Chinese hamster V79 cells**	**LD50 > 1125 mg/kg bw/d** **Negative +/- metabolic act.** **Negative +/- metabolic act.**	**R014584** **C043625** **2013/1224040**
M610F007 5916256 Page 205	**2B**		**Microbial Mutagenicity Assay (Ames)** **In vitro Micronucleus**	**S. typhimurium strains TA98, TA100, TA1535, TA1537 and E.coli WP2 uvrA** **Chinese hamster V79 cells**	**Negative +/- metabolic act.** ***Negative +/- metabolic act.*** **(Preliminary results only)**	**2014/1082362** **2014/1098024** **(assigned docID, study on-going)**

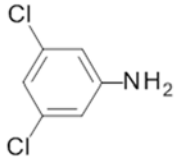
T = Testosterone
act. = activation

Table 5.8.1-1 (continued)

Trivial Name Reg.No.	Group	Structure	Study	Species (Strain)/Test system	Findings	References (BASF DocID)
RP36221 5079618 Page 214	2C		Acute oral toxicity Microbial Mutagenicity Assay (Ames) Mouse Lymphoma (MLTK) In vitro Micronucleus	Wistar rats S. typhimurium strains TA98, TA100, TA1535, TA1537 and E.coli WP2 uvrA Mouse Lymphoma L5178Y cell line Chinese hamster V79 cells	LD50 > 2000 mg/kg bw/d Negative +/- metabolic act. Negative +/- metabolic act. Negative +/- metabolic act.	2013/11688382 2013/1286168 2013/1286166 2013/1164782
RP36115 5079624 Page 247			In vitro mechanistic In vitro AR binding	Porcine Leydig Cell Human mammary gland cancer cell	May inhibit T secretion No AR binding	C029154 R014678
RP36114 5079627 Page 249			In vitro AR binding	Human mammary gland cancer cell	No AR binding	R014678
RP44247 89517 Page 251	2D		Microbial Mutagenicity Assay (Ames) In vitro Micronucleus	S. typhimurium strains TA98, TA100, TA1535, TA1537 and E.coli WP2 uvrA Chinese hamster V79 cells	Negative +/- metabolic act. Negative +/- metabolic act.	2013/1282481 2013/1286160

T = Testosterone
act. = activation

Table 5.8.1-1 (continued)

Trivial Name Reg.No.	Group	Structure	Study	Species (Strain)/Test system	Findings	References (BASF DocID)
RP32596 (3,5-DCA) 85831 Page 271	**3**		**Microbial Mutagenicity Assay (Ames)** **DNA-Repair Assay** **In vivo Micronucleus** **28-day oral gavage**	**S. typhimurium strains TA98** **S. typhimurium strains TA98,TA100** **Primary cultured rat hepatocytes** **NMRI Mice** **Wistar Rats**	**Negative +/- metabolic act.** **Negative +/- metabolic act.** **Negative** **Negative** **NOAEL 7.5 mg/kg bw/d**	**1997/1002678** **1987/1002734** **1988/1003296** **2013/1140617** **2013/1286162**

T = Testosterone
act. = activation

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. Of OASIS TIMES the prediction models for Ames test was considered and therefore predictivity is limited to these test systems only. The reports for the evaluations made are available under DocIDs 2014/1094154, 2013/1414763, and 2013/1414765 in the confidential part, as they also contain information on impurities.
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.

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/] only the two independent statistical prediction models for mutagenicity (Ames) were selected. The data obtained for iprodione and its metabolite can be found in DocIDs 2014/1094158, 2014/1094157 and 2014/1094156 in the confidential part, as they also contain information on impurities. 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.

CAVEAT on reliability of QSAR modules implied
With regard to the QSAR evaluations it should be noted that for nearly all analysis the algorithm reported are out of structural domain. As a consequence the predictivity is solely based on the proposed DNA-interaction via the structural alert, not taking into account possible functional group interaction and stereochemical hindrance. It is well acknowledged that these structural activity predictions are therefore of limited validity. To overcome these limitations the evaluation was conducted mainly in comparison to the parent compound iprodione and metabolites of iprodione where genotoxicity results (Ames test) are available.

Table 5.8.1-2 below summarises the QSAR evaluations conducted:

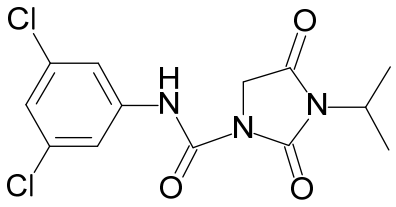
Table 5.8.1-2: Summary of QSAR predictions and study results (Ames test) for plant metabolites of potential human consumer exposure.

		QSAR predicted mutagenicity (Ames)			
		OASIS Times [a,b]		VEGA	
				Caesar (2.1.9)	SarPy (1.0.4-beta)
Substance	**Mutagenicity data (Ames)**	**Result parent**	**Total prediction**	**Prediction**	**Prediction**
RP26019 Reg.No.101169	Negative	Negative; out o.d.[a]	Positive; out o.d.[a]	Non-mutagenic; out o.d.	Non-mutagenic; out o.d.
RP30228 Reg.No. 5079647	Negative	Negative; out o.d.[a]	Positive; out o.d.[a]	Non-mutagenic; out o.d.	Non-mutagenic; out o.d.
RP36112 Reg.No.5079623	Negative	Negative; out o.d.[a]	Positive; out o.d.[a]	Mutagenic; out o.d.	Non-mutagenic; out o.d.
RP32490 Reg.No.5079628	Not available	Negative; out o.d.[a]	Negative; out o.d.[a]	Non-mutagenic; out o.d.	Non-mutagenic; out o.d.
RP25040 Reg.No. 207099	Negative	Positive; out o.d.[a]	Positive; out o.d.[a]	Non-mutagenic; out o.d.	Non-mutagenic; out o.d.
RP37176 Reg.No. 5079612	Negative	Negative; out o.d.[a]	Positive; out o.d.[a]	Non-mutagenic; out o.d.	Non-mutagenic; out o.d.
RP36233 Reg.No.5079632	Not available	Negative; out o.d.[b]	Positive; out o.d.[b]	Non-mutagenic; out o.d.	Non-mutagenic; out o.d.
M610F007 Reg.No.5916256	Negative	Negative; out o.d.[b]	Positive; out o.d.[b]	Non-mutagenic; in domain	Non-mutagenic; in domain
RP36221 Reg.No. 5079618	Negative	Negative; out o.d.[b]	Positive; out o.d.[b]	Mutagenic; out o.d.	non-mutagenic; out o.d.
RP36115 Reg.No.5079624	Not available	Negative; out o.d.[b]	Positive; out o.d.[b]	Mutagenic; out o.d.	Non-mutagenic; out o.d.
RP36114 Reg.No.5079627	Not available	Negative; out o.d.[b]	Positive; out o.d.[b]	Mutagenic; out o.d.	Non-mutagenic; out o.d.
RP44247 Reg.No.89517	Negative	Negative; out o.d.[b]	Positive; out o.d.[b]	Mutagenic; out o.d.	Non-mutagenic; out o.d.
DCHPU Reg.No.5932706	Not available	Negative; out o.d.[b]	Positive; out o.d.[b]	Non-mutagenic; out o.d.	Non-mutagenic; out o.d.
RP32596 Reg.No.85831	Negative	Negative; out o.d.[b]	Positive; out o.d.[b]	Non-mutagenic; in domain	Non-mutagenic; in domain

[a] OASIS TIMES MIX V.2.26.6; Mutagenicity S-9 activated v04.03
[b] OASIS TIMES 2.27.13 TB; Mutagenicity S-9 activated v08.08
o.d. = of domain

In chapter MCA 5.8.1 toxicological relevance assessment for metabolites of potential human consumer exposure, summarized in table Table 5.8.1-1, has been conducted. For each metabolite argumentation for grouping as agreed with the RMS is followed by discussion of QSAR results. This is followed by OECD summaries for acute toxicity, genotoxicity and short-term toxicity where data is available. At the end of each assessment an overall summary and conclusion of the toxicological relevance assessment of the metabolite is given.

Metabolite RP30228 Reg.No. 5079647



RP30228; Reg.No.5079647

RP30228 is a structural isomer of iprodione determined in plant, livestock, rat, soil, water and sediment. Based on close structural relationship between metabolites RP30228 and RP36112 and participation of both metabolites in the same metabolic pathways in plant, livestock and rat, these metabolites are placed into a common assessment group (1A), as agreed with the RMS. As there might be potential human consumer exposure via the food chain this metabolite was considered for toxicological relevance assessment.

Structural Alert for RP30228

OASIS-Times gave no prediction of genotoxicity for parent metabolite RP30228 but an in domain positive prediction after *in silico* metabolism for a DNA-reactive urea-derivative (NC=ON). This hypothetically occurring mutagenic compound potentially emerges after hydrolysis of the amide-group positioned between the dichlorophenyl- and dioxoimidazolidine-ring. However, total predictivity was out of domain mutagen. Similar predictions for hypothetical occurring metabolites with DNA-reactive urea-derivatives are proposed for parent iprodione and metabolite RP25040, which were both negative in the Ames test. This indicates that the identified alert is not relevant.

CAESAR and SarPy predicted RP30228 as non-mutagen potentially out of the applicability domain.

In conclusion in one of the structure activity evaluation tools employed there was a limited alert for genotoxicity *in vitro* after *in silico* metabolic activation which is considered of no relevance based on similar predictions for parent iprodione and metabolite RP25040 for which genotoxicity data indicate non-mutagenicity. Moreover, *in vitro* genotoxicity data for RP30228 also indicates non-mutagenicitgy (BASF DocID C043629).

Acute toxicity studies for RP30228

Report: CA 5.8.1/1
[redacted]., 1976a
30.228 R.P.: Acute toxicity and local tolerance
C022990
Guidelines: none
GLP: no

Executive Summary (Acute toxicity)

Oral: In an acute oral toxicity study 20 CD-1 mice and 20 CD rats (10/sex each) were given a single oral dose via gavage of RP30228 suspended in 10% aqueous solution of gum arabic at a dose level of 4500 and 10000 mg/kg bw for mice and 2500 mg/kg bw for rats.
One female mouse of the 10000 mg/kg bw dose group died on the 3rd day after administration. All other animals of the 10000 mg/kg bw and 4500 mg/kg bw dose groups survived. Furthermore, all rats survived after administration of 2500 mg/kg bw of RP30228 Accordingly, the oral LD_{50} was found to be greater than 10000 mg/kg bw for mice and greater than 2500 mg/kg bw for rats.

Mouse, oral: LD_{50} > 10,000 mg/kg bw
Rat, oral: LD_{50} > 2,500 mg/kg bw

Mice in the highest dose group showed sedation and dyspnea on the day of administration and their mean body weight was found to be slightly reduced compared to the controls. At the dose of 4500 mg/kg bw no symptoms were noted and the weight gain of the mice was entirely normal. No symptoms were noted in the test with the treated rats but the mean body weights of the females were found to be slightly lower than those of the controls during the 15 days of the observation period. Autopsy was done only in rats. No macroscopic anomaly was noted in the organs examined.

Dermal: In an acute dermal toxicity study 20 CD rats (10/sex) were given a single dermal dose via occlusive application for 24 hours. The test substance was suspended with one part ethanol, one part acetone and 2 parts arachis oil. No mortality or any symptoms were noted but the mean body weights of the treated rats were found to be slightly lower than those of the controls. No local cutaneous irritation was observed. No macroscopic anomaly was noted in the organs examined.

Rat, dermal: LD_{50} > 2,500 mg/kg bw

(BASF DocID C022990)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: **1-(3,5-dichlorophenylcarbamoyl)-3-isopropyl dioxoimidazolidine (RP30228)**

Description:	no data
Lot/Batch #:	GD 6590
Purity:	not given
Stability of test compound:	not given

2. Vehicle: acute oral test: 10% aqueous solution of gum arabic
acute dermal test: ethanol (1 part) + acetone (1 part) + arachis oil (2 parts)

3. Test animals:

Species:	Rat and mouse
Strain:	CD rats and CD-1 mice
Sex:	males and females
Age:	not given
Weight at dosing (mean):	20 – 22 g for mice and 160 - 200 g for rats
Source:	Charles River, France
Acclimation period:	not given
Diet:	no data available
Water:	no data available
Housing:	no data available

Environmental conditions:

Temperature:	no data available
Humidity:	no data available
Air changes:	no data available
Photo period:	no data available

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: not given

2. Animal assignment and treatment:

Oral: 20 mice (10 per sex per group) received a single dose of each 4500 or 10000 mg/kg bw of test substance in 10% aqueous solution of gum arabic by oral gavage. Test substance was given to the animals at a volume of 50 mL/kg bw. 20 rats (10 per sex) received a single dose of 2500 mg/kg bw of test substance in 10% aqueous solution of gum arabic by oral gavage using a volume of 5 ml/kg bw.
Simultaneously, 20 mice (10 males and 10 females) and 20 rats (10 per sex), acting as controls, received the vehicle alone under the same conditions.

Dermal: The test substance, a suspension in the vehicle used, was applied to a previously shaved cutaneous area of the back (6x6 cm). The treated area was then covered for 24 hours with an occlusive dressing (aluminium foil + sparadrap). After this interval the dressing was removed and the treated area was washed with tepid soapy water and dried. Simultaneously, 20 untreated rats (10/sex) acting as controls, were shaved and kept under the same condition.
For both tests: The control animals and those treated with the test substance were weighed before treatment and then every 5 days. Autopsy and macroscopic examination (rat only) of the principal thoracic and abdominal organs were carried out on the 15th day of the observation period.

II. RESULTS AND DISCUSSION

A. MORTALITY

Oral: One female mouse died after oral administration of 10000 mg/kg bw of the test substance whereas no deaths occurred in the dose group with 4500 mg/kg bw (see Table 5.8.1-3). Death occurred on the 3rd day of the observation period.
In rats no deaths occurred after oral administration of 2500 mg/kg bw of the test substance.

Dermal: No mortality was observed after dermal administration of 2500 mg/kg bw of the test substance until the end of the observation period.

Table 5.8.1-3: Mortality of mice administered RP30228 by the oral route (gavage)

Sex	Dose (mg/kg bw)	Mortality / animals treated
Females	10000	1/10
Males	10000	0/10
Females	4500	0/10
Males	4500	0/10

B. CLINICAL OBSERVATIONS

Oral: At a dose of 10000 mg/kg bw p.o. mice showed sedation and dyspnea on the day of administration (one female mouse was found dead on the 3rd day after administration). At the dose of 4500 mg/kg bw p.o. no symptoms were noted.
No symptoms were noted in treated rats.

Dermal: No symptoms and no local cutaneous irritation was noted in rats treated dermally with 2500 mg/kg bw for 24 hours.

C. BODY WEIGHT

Oral: In mice, at a dose of 10000 mg/kg bw p.o. the mean weight was found to be slightly (7 to 10%) less than that of the controls after 10 days of the observation period and in females also after 15 days. At the dose of 4500 mg/kg bw p.o. the weight gain of the mice was entirely normal (Table 5.8.1-4).
The mean weights of the female rats were found to be slightly (6 to 7%) lower than those of the controls during the 15 days of the period of observation (Table 5.8.1-5).

Dermal: The mean body weights of treated rats were found to be slightly (5%) lower than those of the controls after 5 or 10 days of the period of observation for females or males, respectively. But the values on the 15th day being very similar to those of the controls (see Table 5.8.1-6).

Table 5.8.1-4: Body weight changes of orally treated mice

Sex	Compound and dose [mg/kg bw]	Initial values [g]	Value after "n" days observation		
			n=5	n=10	n=15
Male	Control 0	21.5±0.2	23.7±0.3	24.3±0.6	24.6±0.4
Female		20.9±0.3	22.1±0.4	22.8±0.5	23.6±0.5
Male	RP30228 10000	21.4±0.3	22.7±0.8	**22.4±0.8[a]**	23.7±0.8
Female		20.5±0.2	21.8±0.5	**20.4±0.8[a]**	**21.8±0.8[a]**
Male	RP30228 4500	21.6±0.2	24.0±0.3	25.0±0.3	25.0±0.6
Female		20.5±0.2	21.3±0.4	22.5±0.5	23.0±0.5

[a] Difference, compared with controls, significant at p = 0.05 (analysis of variance and Students's t-test).

Table 5.8.1-5: Body weight changes of orally treated rats

Sex	Compound and dose [mg/kg bw]	Initial values [g]	Value after "n" days observation		
			n=5	n=10	n=15
Male	Control 0	189.0±2.8	224.5±4.8	251.5±6.5	288.0±7.2
Female		168.0±1.3	186.0±2.2	197.0±3.7	208.5±3.4
Male	RP30228 2500	190.0±3.0	225.5±4.0	262.5±4.8	284.5±4.8
Female		165.0±1.7	**175.5±1.7[a]**	**185.5±1.6[b]**	**195.0±2.3[a]**

[a] Difference, compared with controls, significant at p = 0.05 (analysis of variance and Students's t-test).
[b] Difference, compared with controls, significant at p = 0.01 (analysis of variance and Students's t-test).

Table 5.8.1-6: Body weight changes of dermally treated rats

Sex	Compound and dose [mg/kg bw]	Initial values [g]	Value after "n" days observation		
			n=5	n=10	n=15
Male	Control	168.0±1.8	206.0±3.3	248.0±4.6	275.0±3.9
Female	0	172.0±2.5	192.5±3.6	210.0±3.6	215.0±3.4
Male	RP30228	169.5±2.2	196.0±2.3	**235.5±3.7**[a]	267.5±3.5
Female	2500	172.0±2.9	**183.5±2.9**[a]	205.5±2.9	213.0±4.3

[a] Difference, compared with controls, significant at p = 0.05 (analysis of variance and Students's t-test).

D. NECROPSY

An autopsy was only performed in rats. No macroscopic anomaly was noted in the organs examined after oral or dermal treatment.

III. CONCLUSION

Under the experimental conditions of this study the oral LD_{50} of RP30228 in mice was determined to be greater than 10000 mg/kg bw and in rats greater than 2500 mg/kg bw. The dermal LD_{50} was determined to be greater than 2500 mg/kg bw.

Executive Summary (Local tolerance)

The local ocular and cutaneous tolerance was tested in 6 New Zealand White rabbits each. For the ocular tolerance test 100 mg undiluted RP30228 was applied to the conjuctival sac of the left eye of each rabbit. The evaluation and interpretation of the ocular irritation was done after 1 hour and 1, 2, 3, 4 and 7 days after application. No opacity of the cornea, no effect on the iris, no edema of the conjunctiva and no tear secretion were noted in the six treated rabbits 1, 2, 3, 4 and 7 days after application. Individual scores were not given in the study report. For the cutaneous tolerance test 500 mg moistened RP30228 was applied for 24 hours to intact or scarified skin under occlusive conditions. Local reactions were evaluated after 24 and 72 hours after application. No irritant action on intact or scarified skin was noted. Individual skin reading scores were not given in the study report. Data on body weight development or necropsy were not given in the study report. Based on the findings of this study RP30228 does not show an eye and skin irritation potential.

(BASF DocID C022990)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: **1-(3,5-dichlorophenylcarbamoyl)-3-isopropyl dioxoimidazolidine (RP30228)**

Description:	no data
Lot/Batch #:	GD 6590
Purity:	not given
Stability of test compound:	not given

2. Vehicle: none, pure test substance was used

3. Test animals:

Species:	rabbit
Strain:	New Zealand white
Sex:	not given
Age:	not given
Weight at dosing (mean):	2.5 to 3.0 kg
Source:	not given
Acclimation period:	not given
Diet:	no data available
Water:	no data available
Housing:	no data available

Environmental conditions:

Temperature:	no data available
Humidity:	no data available
Air changes:	no data available
Photo period:	no data available

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: not given

2. Animal assignment and treatment:

Ocular: The test is based on the protocol of Draize. One application of 100 mg of the pure test substance was applied into the conjunctival sac of the left eye of each of rabbit. Six rabbits were used for this experiment. Evaluation and interpretation of the ocular irritation was done after one hour and after 1, 2, 3, 4 and 7 days after application.

Cutaneous: The test is based on the "Patch-test technique" of Draize. Six white rabbits were shaved. The left site was left intact while the right site was scarified. 500 mg pure test substance (moistened with a little water) was applied to the intact or scarified skin, respectively. The test substance was covered by small aluminum discs of about 3 cm diameters which again was covered by gauze and held in place by adhesive tape and a bandage encircling the trunk of the animals. The test substance was left on the skin for 24 hours. The skin was observed after 24 hours after application (i.e. the time when the discs are removed) and 72 hours after application.

II. RESULTS AND DISCUSSION

Ocular: RP30228 applied as the pure test substance is devoid of irritant action: no opacity of the cornea, no effect on the iris, no edema of the conjunctiva and no tear secretion were noted in the 6 treated rabbits 1, 2, 3, 4, and 7 days after application. During the first hours of observation a slight redness of the palpebral conjunctiva was observed. This is very probably related to a mechanical irritation since the compound was applied in form of a powder.

Cutaneous: No irritant action on intact or scarified skin was noted.

III. CONCLUSION

Under the experimental conditions of this study the pure test substance did not cause a local irritation on the eye and on intact or scarified skin.

Assessment of acute toxicity for RP30228

Acute oral toxicity studies in rats and mice as well as acute dermal toxicity study in rats demonstrate low toxicity. No potential for eye- or dermal irritation was observed.

Genotoxicity studies for RP30228

Report: CA 5.8.1/2
Molinier B., 1992a
Reverse mutation assay by the AMES test - RP 30228
C043629

Guidelines: OECD 471, US EPA (1984) Pesticide Assessment Guidelines. Subdiv. F - Hazard Evaluation: Human and domestic animals (Nov 1984 revised edition)

GLP: yes

Executive Summary

S. typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 were exposed to RP30228 (metabolite of BAS 610 F; Batch: EA2025RF4; Purity: 99.3%) using dimethylsulfoxide (DMSO)-Tween 80 as a solvent in the presence and absence of metabolic activation in two independent plate incorporation experiments. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment. Bacteriotoxicity was investigated prior to the main experiments in TA 100 at concentrations of 10, 50, 100, 500, 1000 and 2000 µg/plate. Slight toxicity was observed at 1000 and 2000 µg/plate. Concentrations of 125, 250, 500, 1000 and 2000 µg/plate were used in both plate incorporation assays with and without metabolic activation, respectively. Precipitation was observed at and above 500 µg/plate in the tests with and without metabolic activation. 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 RP30228 is not mutagenic in the Salmonella typhimurium reverse mutation assay under the experimental conditions of the study.

(BASF DocID C043629)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material RP30228 (Metabolite of BAS 610 F)

Description: Powder, white

Lot/Batch #: EA2025RF4

Purity: 99.3%

Stability of test compound: Stable in solvent over 4 hours (determined by HPLC/UV)

Solvent used: Dimethylsulfoxide (DMSO) with 20% Tween; after adding the test substance to the solvent, the mixture was heated at about 45°C under shaking for 45 minutes.

2. Control Materials:

Negative control: Spontaneous revertants of each strain alone were determined in the experimental part without metabolic activation.

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.

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
All strains	2-anthramine (2AM)	DMSO	2 µg/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	Sodium azide (NaN_3)	DMSO	1 µg/plate
TA 1535			
TA 1537	9-amino-acridine (9AA)	DMSO	50 µg/plate
TA 1538	2-nitrofluorene (2NF)	DMSO	0.5 µg/plate
TA 98			

3. Activation: S9 was produced from the livers of induced male Sprague-Dawley rats. The S9 was purchased from Iffa Credo (L'Arbresle, France).

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
S9	10 %

4. Test organisms: S. typhimurium strains: TA98, TA100, TA1535, TA1537, TA1538

5. Test concentrations:

Plate incorporation assay: In two independent assays, triplicate plates were prepared for each concentration (vehicle control; 125, 250, 500, 1000 and 2000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains indicated above.

B. TEST PERFORMANCE:

1. Dates of experimental work: 19-May-1992 to 09-Jul-1992

2. Plate incorporation assay:

The test substance solution, of which the volume varied between 50 and 200 µL/plate, 0.5 mL of phosphate buffer (assays without S9 mix) or of S9 mix (assays with S9 mix) and 0.1 mL of the strain were added to 2 mL molten agar containing traces of histidine and biotin and maintained at +45°C. After rapid homogenization, the mixture was spread out on a Petri plate containing minimum medium. After 48 to 72 hours of incubation at 37°C, revertants were scored with an automatic counter.

2. Toxicity test:

In order to determine the maximum concentration of the test substance which does not modify the bacterial growth, 10, 50, 100, 500, 1000 and 2000 µg/plate were tested in the TA 100 strain. If toxicity occurred, the background lawn was sparse compared to control plates and/or the number of colonies had decreased. The sterility of the test substance was checked during this assay.

3. Statistics:

No special statistical tests were performed.

4. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- if each test induces a doubling in the number of revertants when compared to that in the negative and/or solvent controls, for at least one of the tested strains and at one or more of the tested concentrations. In this case, a statistically significant dose relationship is investigated, using a linear regression analysis, and considered as significant if $p \leq 0.05$ (for $n = 18$ values, the correlation coefficient r must be ≥ 0.47).

A test substance is generally considered non-mutagenic in this test if:

- if the above two criteria are not fully met

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance in the vehicle DMSO over a period of 4 hours was verified by HPLC/UV analysis.

B. TOXICITY

No toxicity of the test substance was observed in the TA 100 strain with and without S9 mix. Only without S9 mix, in the assay without phosphate buffer, slight toxicity was observed at 1000 and 2000 µg/plate.
A slight precipitate of the test substance was observed at the concentrations ≥ 500 µg/plate without S9 mix, but not with S9 mix. This difference was first attributed to the fact that in the assays with S9 mix, the test substance solution was more diluted (due to the addition of 0.5 mL S9 mix). Therefore, in the second toxicity test without S9 mix 0.5 mL phosphate buffer was added to dilute the substance solution. But in that case, the precipitate was always observed at 500 µg/plate.

C. MUTATION ASSAYS

No biologically relevant increase in number of revertants was observed in any strain tested neither with nor without metabolic activation (see Table 5.8.1-7). Precipitation was observed at and above 500 µg/plate with and without metabolic activation.

Table 5.8.1-7: Bacterial gene mutation assay with RP30228 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
1st Plate incorporation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
RS	14	14	72	70	8	9	3	5	8	13
Neg. control (Tween 80 - DMSO)		11		68		9		5		7
RP30228										
125 µg/plate	12	11	75	76	7	10	4	6	5	7
250 µg/plate	11	13	76	64	11	12	5	7	7	7
(P)500 µg/plate	15	11	71	75	10	10	6	6	9	7
(P)1000 µg/plate	12	11	72	65	13	9	4	4	8	8
(P)2000 µg/plate	10	12	53	63	11	7	5	6	8	4
Pos. control§	630	115	1001	440	179	267	118	313	605	191
2nd Plate incorporation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
RS	14	15	76	102	13	12	4	7	8	12
Neg. control (Tween 80 - DMSO)		10		71		12		6		9
RP30228										
125 µg/plate	12	13	104	82	11	15	7	6	10	9
250 µg/plate	15	11	78	76	12	13	7	7	8	6
(P)500 µg/plate	15	15	96	83	11	12	4	7	12	10
(P)1000 µg/plate	19	11	83	84	11	14	6	10	8	8
(P)2000 µg/plate	11	7	80	90	9	12	6	6	7	7
Pos. control§	1186	136	1752	447	220	319	157	270	1295	196

§ = Compound and concentrations see Material and Methods (I.A.2.) above
RS= spontaneous revertants (only conducted in the experimental part without metabolic activation)

III. CONCLUSION

According to the results of the present study, the test substance RP30228 is <u>not</u> mutagenic in the Salmonella typhimurium reverse mutation assay under the experimental conditions chosen here.

Report:	CA 5.8.1/3 Bohnenberger S., 2013c Reg.No. 5079647 (metabolite of BAS 610 F, Iprodione) - In vitro micronucleus test in Chinese hamster V79 cells 2013/1247542
Guidelines:	OECD 487 (2010), Commission Regulation EU No. 640/2012 of 06 July 2012 - B.49: In vitro Mammalian Cell Micronucleus Test
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

Reg. No. 5079647 (Batch DP646B; purity 99.8%) was tested in vitro for its potential to induce micronuclei in V79 cells of the Chinese hamster in the absence and presence of metabolic activation by S9 mix in four independent experiments. Concentrations of 0.2 to 2040 µg/mL were tested within an exposure period of 4 hours in a pre-test with and without metabolic activation. Since no cytotoxicity was observed, this pre-test was designated Experiment I and 2040.0 µg/mL was chosen as highest applied concentration for all subsequent experiments. In Experiment IIA the exposure period was 24 hours without metabolic activation and 4 hours with metabolic activation. In Experiment IIB the exposure period was 4 hours with metabolic activation. In Experiment IIC the exposure period was 24 hours without metabolic activation. Visible precipitation of the test item in the culture medium was observed microscopically at the end of treatment in the absence and presence of S9 mix at 3.3 µg/mL and above. In Experiment I in the absence of S9 mix after treatment with 52.2 and 326.4 µg/mL slight statistically significant increases in micronucleated cells were observed, which were considered as biologically irrelevant, since the number of micronucleated cells was in the range of the laboratory historical control data. In Experiment IIA in the absence of S9 mix (24 hours exposure) one single statistically significant increase in micronucleated cells was observed after treatment with 326.4 µg/mL being slightly above the range of the laboratory historical control data. In Experiment IIC in the absence of S9 mix (24 hours exposure) all evaluated concentrations showed statisitically significant and dose-dependent increases in micronucleated cells with the two highest values exceeding the range of the laboratory historical control data. In Experiment I in the presence of S9 mix no increase in micronucleated cells was observed. In Experiment IIA in the presence of S9 mix statistically significant increases in micronucleated cells clearly exceeding the range of the laboratory historical control data were observed after treatment with 0.5 and 1.3 µg/mL. In Experiment IIB with S9 mix these findings could not be confirmed. Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test. Based on the results of this study Reg. No. 5079647 is considered to induce micronuclei *in vitro* in V79 cells in the presence or absence of metabolic activation.

(BASF DocID 2013/1247542)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg. No. 5079647 (Metabolite of BAS 610 F, iprodione)

Description:	Solid, white
Lot/Batch #:	DP646B
Purity:	99.8 % (tolerance ± 1.0 %)
Stability of test compound:	Stable in DMSO (solvent)
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control:	A negative control was not employed in this study
Solvent control:	DMSO
Positive controls, -S9:	Mitomycin C (MMC, 0.3 µg/mL, dissolved in deionised water) Griseofulvin (8.0 µg/mL, dissolved in DMSO)
Positive control, +S9:	Cyclophosphamide (CPA, 20.0 µg/mL (Exp. I), 15.0 µg/mL (Exp. IIA and IIB), dissolved in saline)

3. Activation: S9 was produced from male Wistar rats. The rats were induced by intraperitoneal applications of 80 mg/kg bw phenobarbital and by oral administrations of 80 mg/kg bw β-naphthoflavone each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g. Aliquots of the supernatant were frozen and stored in ampoules at –80 °C. The S9 mix preparation was performed according to Ames et al. Briefly, an appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution (see below) to result in a final protein concentration of 0.75 mg/mL in the cultures. During the experiment the S9 mix was stored on ice.

The S9-mix was prepared immediately before use and had the following composition:

Component	*Concentration*
Sodium-ortho-phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

4. Test organisms: Chinese hamster V79 cells

This is a continuous cell line with a population doubling time of 13 hours.

5. Culture medium/conditions: About 5 x 10^5 cells/flask were seeded in 15 mL of MEM (minimal essential medium) containing Hank's salts, glutamine, Hepes (25 mM), penicillin/ streptomycin (100 U/mL/100 mg/mL) and 10 % (v/v) fetal bovine serum (FBS). Cell cultures were incubated at 37 °C in a humidified atmosphere with 1.5% carbon dioxide (98.5% air).

Exponentially growing stock cultures more than 50 % confluent were rinsed with Ca-Mg-free salt solution. Afterwards the cells were treated with trypsin-EDTA-solution at 37 °C for approx. 5 minutes. By adding complete culture medium including 10 % (v/v) FBS the enzymatic treatment was stopped and a single cell suspension was prepared. The cells were seeded into Quadriperm dishes, which contained microscopic slides. Into each chamber 1.0 x 10^5 – 1.5 x 10^5 cells were seeded with regard to the preparation time. In each experimental group two parallel cultures were set up.

6. Test concentrations:

a) Preliminary toxicity assay: 0.2 - 2040.0 µg/mL with and without metabolic activation

Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I (see Table 5.8.1-8).

b) Cytogenicity assay: Experiment IIA:

0.2 - 2040.0 µg/mL without metabolic activation

0.5 - 2040.0 µg/mL with metabolic activation

Experiment IIB:

0.13 - 20.0 µg/mL with metabolic activation

Experiment IIC:

150.5 - 450.0 µg/mL without metabolic activation

Table 5.8.1-8: Doses applied in the Micronucleus Test in Chinese Hamster V79 Cells with Reg.No. 5079647 (Metabolite of BAS 610 F, iprodione)

Preparation interval	Exposure period	Exp.	Concentration in µg/mL								
			Without S9 mix								
24 hrs	4 hrs	I	0.2	0.5	**1.3**	**3.3^{P}**	8.4^{P}	20.9^{P}	**52.2^{P}**	**130.6^{P}**	**326.4^{P}**
										816.0^{P}	2040.0^{P}
24 hrs	24 hrs	IIA	0.2	0.5	**1.3**	**3.3^{P}**	8.4^{P}	20.9^{P}	52.2^{P}	**130.6^{P}**	**326.4^{P}**
										816.0^{P}	2040.0^{P}
24 hrs	24 hrs	IIC			150.0^{P}	200.0^{P}	**250.0^{P}**	**300.0^{P}**	**350.0^{P}**	400.6^{P}	450.0^{P}
			With S9 mix								
24 hrs	4 hrs	I	0.2	0.5	**1.3**	**3.3**	**8.4^{P}**	20.9^{P}	52.2^{P}	130.6^{P}	**326.4^{P}**
										816.0^{P}	2040.0^{P}
24 hrs	4 hrs	IIA		**0.5**	**1.3**	**3.3^{P}**	8.4^{P}	20.9^{P}	52.2^{P}	130.6^{P}	326.4^{P}
										816.0^{P}	2040.0^{P}
24 hrs	4 hrs	IIB	0.13	**0.25**	**0.50**	**0.75**	1.0	**1.25**	**1.5**	**5.0^{P}**	10.0^{P}
											20.0^{P}

Evaluated experimental points are shown in bold characters

^{P} Precipitation occurred at the end of treatment

B. TEST PERFORMANCE:

1. Dates of experimental work: 23-Jan-2013 - 26-Jun-2013

2. Preliminary cytotoxicity assay: With respect to the solubility of the test item, 2040.0 µg/mL of Reg. No. 5079647 was applied as top concentration for treatment of the cultures in a pre-test. Test item concentrations between 0.2 and 2040.0 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity.

3. Cytogenicity Assay:

Exposure period 4 hours: The culture medium of exponentially growing cell cultures was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL medium was added.

Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed twice 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. Then the cells were cultured in complete medium containing 10% (v/v) FBS for the remaining culture time of 20 hours.

Exposure period 24 hours: The culture medium of exponentially growing cell cultures was replaced with complete medium containing 10% (v/v) FBS including the test item without S9 mix. The medium was not changed until preparation of the cells. Concurrent solvent and positive controls were performed.

Preparations of cultures: For the micronucleus analysis, 24 hours after the start of the exposure, the cells were treated on the slides in the chambers of the quadriperm dishes with deionised water for 1 to 1.5 min at 37 °C. Afterwards the cells were fixed twice with a solution containing 3 parts ethanol, 1 part acetic acid and 1.25 % (v/v) formaldehyde. After preparation the cells were stained with Giemsa and labelled with a computer-generated random code to prevent scorer bias.

Analysis of micronuclei and cytotoxicity:

Evaluation was performed manually using microscopes with 40x objectives. The micronuclei were stained in the same way as the main nucleus. The area of the micronucleus did not extend the third part of the area of the main nucleus. 1000 cells in two parallel cultures were scored for micronuclei, so that at least 2000 cells from clones with 2 - 8 cells were analysed per test group. The frequency of micronucleated cells was reported as % micronucleated cells.

Cytotoxicity was assessed via counting the number of clones consisting of 1 cell (c1), 2 cells (c2), 3 - 4 cells (c4), and 5 - 8 cells (c8) among the cells that were scored for the presence of micronuclei. These clusters represented the cells that have divided 1, 2, or 3 times within the experiment. From these data, a proliferation index (PI) was calculated (see formula below). Only those cultures were evaluated which showed a PI > 1.3, in order to guarantee for a sufficient cell proliferation during treatment and recovery.

$$PI = \frac{(c1 \times 1) + (c2 \times 2) + (c4 \times 3) + (c8 \times 4)}{(c1 + c2 + c4 + c8)}$$

PI: Proliferation index
cx: Number of clones with x cells (with x: 1, 2, 4, or 8)

4. Statistics:

Statistical significance at the five per cent level was evaluated by means of the Chi-square test. Evaluation was performed only for test groups showing a higher number of micronucleated cells than the respective solvent control group

5. Evaluation criteria:

A test item was classified as mutagenic if:

- the number of micronucleated cells is not in the range of the historical 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.

A test item can be classified as non-mutagenic if:

- the number of micronucleated cells in all evaluated test groups is in the range of the historical control data and
- no statistically significant or concentration-related increase in the number of micronucleated cells is observed in comparison to the respective solvent control.

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 was verified analytically ([see 2014/1095063 Becker M.,Kamp H. 2013 a]) as was the homogeneous distribution in vehicle ([see 2014/1095066 Becker M.,Kamp H. 2014 a]).

B. PRELIMINARY CYTOTOXICITY ASSAY:

Since the cultures fulfilled the requirements for cytogenetic evaluation, the preliminary test was designated Experiment I (see below). In the pre-test and in all subsequent experimental parts in the absence and presence of S9 mix no cytotoxicity was observed at the evaluated concentrations.

C. CYTOGENICITY ASSAYS:

Four independent experiments were performed. In Experiment I the exposure period was 4 hours with and without metabolic activation. In Experiment IIA the exposure period was 24 hours without metabolic activation and 4 hours with metabolic activation. In Experiment IIB the exposure period was 4 hours with metabolic activation. In Experiment IIC the exposure period was 24 hours without metabolic activation. The cells were prepared 24 hours after start of treatment with the test item.

Visible precipitation of the test item in the culture medium was observed microscopically at the end of treatment in Experiment I in the absence of S9 mix and in Experiment IIA in the absence and presence of S9 mix at 3.3 µg/mL and above and in Experiment I at 8.4 µg/mL and above in the presence of S9 mix. In Experiment IIB in the presence of S9 mix precipitation was observed at 5.0 µg/mL and above and in Experiment IIC in the absence of S9 mix at 150.0 µg/mL and above microscopically at the end of treatment.

In Experiment I in the absence of S9 mix after treatment with 52.2 and 326.4 µg/mL, slight statistically significant increases in micronucleated cells (1.50 and 1.00 %) were observed (see Table 5.8.1-9). These findings were regarded as biologically irrelevant since the number of micronucleated cells were in the range of the laboratory historical control data (0.15 - 1.50 % micronucleated cells). In Experiment IIA in the absence of S9 mix (24 hours exposure) one single statistically significant increase in micronucleated cells (1.63 %) was observed after treatment with 326.4 µg/mL being slightly above the range of the laboratory historical control data without S9 mix (0.05 - 1.50 % micronucleated cells).
In Experiment IIC in the absence of S9 mix (24 hours exposure) all evaluated concentrations (250.0, 300.0 and 350.0 µg/mL) showed statisitically significant and dose-dependent increases in micronucleated cells (1.48, 1.55 and 1.79 % micronucleated cells). The two highest values exceeded the range of the laboratory historical control data (0.05 - 1.50 % micronucleated cells). In Experiment I in the presence of S9 mix no increase in micronucleated cells was observed. In Experiment IIA in the presence of S9 mix, statistically significant increases in micronucleated cells (5.90 and 2.20 %) clearly exceeding the range of the laboratory historical control data (0.05 - 1.70 % micronucleated cells) were observed after treatment with 0.5 and 1.3 µg/mL. In Experiment IIB with S9 mix these findings could not be confirmed.

Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test.

Table 5.8.1-9: Summary of results of micronucleus test with Reg. No. 5079647 (Metabolite of BAS 610 F, iprodione)

Exp.	Preparation interval	Test item concentration [µg/mL]	Proliferation index	Micronucleated Cells* [%]
Exposure period 4 hrs without S9 mix				
I	24 hrs	Solvent control[1]	2.80	0.45
		Positive control[2]	2.40	**7.90[S]**
		1.3	2.91	0.20
		3.3[P]	2.89	0.35
		52.5[P]	2.60	**1.50[S]**
		130.6[P]	2.54	0.90
		326.4[P]	2.53	**1.00[S]**
Exposure period 24 hrs without S9 mix				
IIA	24 hrs	Solvent control[1]	2.81	0.45
		Positive control[3]	2.71	**11.10[S]**
		1.3	2.90	0.35
		3.3[P]	2.93	0.15
		130.6[P]	2.51	0.90
		326.4[P]**	2.84	**1.63[S]**
IIC	24 hrs	Solvent control[1]	2.97	0.45
		Positive control[3]	2.58	**8.95[S]**
		250.0[P]**	2.65	**1.48[S]**
		300.0[P]**	2.72	**1.55[S]**
		350.0[P]**	2.95	**1.79[S]**
Exposure period 4 hrs with S9 mix				
I	24 hrs	Solvent control[1]	2.05	0.55
		Positive control[4]	1.51	**4.75[S]**
		1.3	2.06	0.15
		3.3	2.15	1.05
		8.4[P]	2.13	0.20
		326.4[P]	2.01	n.e.
IIA	24 hrs	Solvent control[1]	2.16	1.05
		Positive control[5]	1.50	**3.15[S]**
		0.5	1.55	**5.90[S]**
		1.3	2.26	**2.20[S]**
		3.3[P]	2.34	1.00
		816.0[P]	1.86	n.e.
IIB	24 hrs	Solvent control[1]	1.97	1.45
		Positive control[5]	1.59	**12.30[S]**
		0.25	1.86	0.35
		0.50	1.54	0.40
		0.75	1.90	0.40
		1.25	1.97	1.05
		1.50	1.83	0.85
		5.00[P]	1.82	0.65

* The total number of micronucleated cells was determined in a sample of 2000 cells
n.e. Not evaluated since the highest applied concentration is not cytotoxic
[S] Number of micronucleated cells statistically significantly higher than corresponding control values
[P] Precipitation occurred microscopically at the end of treatment
[1] DMSO 0.5 % (v/v)
[2] Mitomycin C 0.3 µg/mL
[3] Griseofulvin 8.0 µg/mL
[4] CPA 20.0 µg/mL
[5] CPA 15.0 µg/mL

III. CONCLUSIONS

Based on the results of the study it is concluded that Reg. No. 5079647 is considered as mutagenic since it induced micronuclei in V79 cells in the presence or absence of metabolic activation under the test conditions.

Report:	CA 5.8.1/4 [redacted]., 2014a Reg. No. 5079647 (metabolite of BAS 610 F, Iprodione) - Micronucleus assay in bone marrow cells of the mouse, oral administration 2013/1286156
Guidelines:	OECD 474 (1997), (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.12, EPA 870.5395
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

Reg.No. 5079647 metabolite of BAS 610 F (iprodione, Batch: DP646B, Purity: 99.8%) was tested for the ability to induce micronuclei in the bone marrow of NMRI mice. For this purpose, the test substance suspended in corn oil was administered once orally to groups of 7 male mice at a dose level of 2000 mg/kg body weight in a volume of 20 mL/kg body weight. Based on formulation analysis results the achieved mean dose level was calculated to be 1526 mg/kg bw. The animals were sacrificed 24 or 48 (additional high dose and vehicle group (5 animals/group) hours after the administration and the bone marrow of the femora was prepared for micronuclei analysis. The vehicle served as negative and cyclophosphamide as positive control. After staining of the preparations, 6000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei (except for the positive control group, for which only 2000 PCEs were scored). To investigate a cytotoxic effect due to the treatment with the test item the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and reported as the number of PCEs per 2000 erythrocytes. No clinical symptoms related to the test item were observed. Treatment with Reg.No. 5079647 not substantially decreased the number of PCEs as compared to the mean value of PCEs of the vehicle. Plasma analysis perfomed with samples taken 24 hours after application of the test item demonstrated that all mice were systemically exposed to the test item, confirming the validity of the outcome of this study. No biologically relevant increase in the frequency of micronuclei was observed at any preparation interval. A marginally higher micronucleus frequency showing statistical significance (p=0.0455) occurred in the 24 h preparation interval dose group in comparison with the concurrent vehicle group. However, this was considered to be incidental and of no biological relevance at all, since the values obtained (both mean and individual) were within the range for historical vehicle controls. In addition, on an individual animal basis, the number of micronucleated cells per 2000 PCEs in one control animal was above the highest occurrence of micronucleated cells in any of the test substance treatment groups. The positive control cyclophosphamide led to the expected increase of micronuclei frequency, thus demonstrating the sensitivity of the test system. Based on the results of this study Reg. No. 5079647 is not considered to induce micronuclei in the bone marrow cells of mice.

(BASF DocID 2013/1286156)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Reg.No. 5079647 (Metabolite of BAS 610 F, iprodione)
Description:	Solid, white
Lot/Batch #:	DP646B
Purity:	99.8 % (tolerance ± 1.0%)
Stability of test compound:	The stability of the test substance in the solvent was confirmed indirectly by dose formulation analytics ([see 2014/1095066 Becker M.,Kamp H. 2014 a])
Vehicle used:	Corn oil
2. Control Materials:	
Negative:	No negative control was employed in this study.
Vehicle control:	Corn oil
Positive control:	Cyclophosphamide (CCP) 40 mg/kg bw, dissolved in sterile water
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 weight at dosing:	37.6 g
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	
Range finding study:	2/sex
Micronucleus assay:	7 males/dose (exception for vehicle and positive control groups: 5 animals each)
Acclimation period:	At least 5 days
Diet:	Pelleted standard diet, ad libitum
Water:	Tap water, ad libitum
Housing:	Individually in Makrolon cages, type II/III, with wire mesh top

4. Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	45 - 65%
Air changes:	frequency not indicated
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:	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: 24-Jul-2013 to 02-Sep-2013

2. Preliminary range finding test:

Male and female 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 7 male mice were treated once by oral gavage with 2000mg Reg. 5079647/kg bw. Five mice were concomitantly treated with either the vehicle or the positive control. 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. As only a homogeneous suspension and not a clear solution was achieved in the vehicle, samples and corresponding reserve samples of top/middle/bottom of all dose formulations and of the vehicle control (1 mL, each) were taken on the first treatment day immediately after the last application of the test item and stored at ≤ - 18 °C at Harlan CCR until shipment to the sponsor who performed a separate analytical study ([see 2014/1095066 Becker M.,Kamp H. 2014 a]). The animals were observed for evident clinical signs of toxicity throughout the study.

	Twenty-four hours after the administration the mice were sacrificed, blood samples taken for further analysis as separate study contracted by the sponsor (see BASF DocID 2013/1286156). Than the femora were removed, the epiphyses were cut off and the marrow was flushed out with foetal calf serum using a syringe. The cell suspension was centrifuged at 1500 rpm (390 x g) for 10 minutes and the supernatant was discarded. 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:	A small drop of the re-suspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald/Giemsa. Cover slips were mounted with EUKITT. At least one slide was made from each bone marrow sample.
Slide evaluation:	Evaluation of the slides was performed using microscopes with 100x oil immersion objectives. Per animal 6000 polychromatic erythrocytes (PCE) were analysed for micronuclei, except for the positive control group, for which only 2000 polychromatic erythrocytes (PCE) were analysed per animal. To investigate a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 2000 erythrocytes. The analysis was performed with coded slides.

4. Statistics:

The number of polychromatic erythrocytes with micronuclei was analyzed by comparing the dose groups with the vehicle control using the non-parametric Mann-Whitney test at the five per cent level ($p < 0.05$).

5. Evaluation criteria:

A test item was considered 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 were used as an aid in evaluating the results, if necessary. However, the primary point of consideration was the biological relevance of the results.
A test item that failed to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes was considered non-mutagenic in this system.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance in the vehicle corn oil was verified analytically. Formulation analysis of the test item treatment formulation revealed a mean concentration of 76.3 % of the nominal concentration which would correspond to a dose level of 1526 mg/kg b.w. ([see 2014/1095066 Becker M.,Kamp H. 2014 a])
Plasma analysis revealed the presence of test item in samples of all mice of the 24-hour test item group (and absence of test item in all control mice, limit of detection 1.0 ng/mL) demonstrating that all these mice were systemically exposed to the test item even 24 hours after application (BASF DocID 2013/1286156).

B. PRELIMINARY RANGE FINDING TEST

No death were observed after administration of 2000 mg/kg bw to male and female animals. Soft feces were the only clinical sign transiently observed in all four animals. This effect was considered to be caused by the high vehicle volume previously also seen in studies performed with the same corn oil volume. Since other vehicles tested in advance did not provide an appropriate formulation, corn oil was chosen to also be used as vehicle in the main experiment.

C. MICRONUCLEUS ASSAY

As previously observed in the pre-test, the only clinical sign transiently observed was soft feces in all animals. In comparison to the corresponding vehicle controls, there was no biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval after oral administration of 2000mg (1526 mg/kg bw/d based on formulation analysis) Reg. 5079647/kg bw (see Table 5.8.1-10). A marginally higher micronucleus frequency showing statistical significance occurred in the 24 h preparation interval dose group in comparison with the concurrent vehicle group. However, this was considered to be incidental and of no biological relevance at all, since the values obtained (mean frequency 0.107%, individual range: 1-3) were perfectly within the range for historical vehicle controls (mean frequency 0.107±0.081, range of mean group values: 0 – 0.450, individual range: 0-9). In addition, on an individual animal basis, the number of micronucleated cells per 2000 PCEs in one control animal was above the highest occurrence of micronucleated cells in any of the test substance treatment groups.

After treatment with the test item the number of PCEs was not substantially decreased as compared to the mean value of PCEs of the vehicle control thus indicating that Reg.No. 5079647 did not exert a cytotoxic effect in the bone marrow. Plasma analysis perfomed with samples taken 24 hours after application of the test item demonstrated that all these mice were systemically exposed to the test item confirming the validity of the outcome of this study.

The positive control cyclophosphamide demonstrated the sensitivity of the test system by inducing micronuclei in PCEs (2.64%).

Table 5.8.1-10: Micronucleus test in mice administered Reg. No. 5079647 by oral gavage

Test group	Dose (mg/kg bw)	Sampling time (h)	PCEs with micronuclei (%)	Range	PCE per 2000 erythrocytes
Vehicle	0	24	0.050	0 - 2	1153
Test item	2000 (1526)[$]	24	**0.107***	1 - 3	1166
Positive control	40	24	**2.640***	34 – 69	1152
Vehicle	0	48	0.100	1 – 4	1213
Test item	2000 (1526)[$]	48	0.107	1 - 3	1165

[$] Dose level calculated on the basis of formulation analysis results
* Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of the non-parametric Mann-Whitney test

III. CONCLUSION

Based on the result of this study, Reg. No. 5079647 does not induce the formation of micronuclei in mouse polychromatic erythrocytes under the conditions of the study.

Assessment of genotoxicity for RP30228

RP30228 does not cause point mutations in bacterial cells but was tested positive *in vitro* in a micronucleus test. As agreed with the RMS, an *in vivo* micronucleus test was conducted showing that RP30228 does not induce micronuclei in mice bone marrow cells. By weight of evidence RP30228 is not considered to be genotoxic.

Sub-chronic toxicity of RP30228

Report: CA 5.8.1/5
[redacted]., 1993a
13-week toxicity study by oral route (dietary admixture) in rats - RP 30228
C043621
Guidelines: OECD 408, FIFRA 40 CFR 160
GLP: yes

Executive Summary

Sprague-Dawley rats (10/sex/group) received RP30228 (batch: EA2025RF4; purity: 99.3%) in their feed for a period of 13 weeks at dose levels of 815, 3250 and 13000 ppm, corresponding to 58, 234 and 954 mg/kg bw/day for males and 64, 252 and 1025 mg/kg bw/day for females. During the study period no mortality and treatment-related clinical signs were observed. Food consumption of all treated animals was similar to that of the respective controls. In treated males, efficiency of food utilization was also similar to that of controls. In treated females, efficiency of food utilization was slightly lower to that of controls. Consequently, the body weight gain of all treated males was similar to that of controls but a lower body weight gain was observed in females given 13000 ppm. There were no treatment-related changes observed at ophthalmology, haematology, blood chemistry and urinalysis. Effects observed at gross pathology and histopathology were considered not treatment related. Therefore, the no observed adverse effect level (NOAEL) under the conditions of the present study was determined to be 3250 ppm, which is equivalent to about 234 mg/kg bw/d in males and 252 mg/kg bw/d in females.

(BASF DocID C043621)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: RP30228

Description:	Powder, white
Lot/Batch #:	EA2025RF4
Purity:	99.3%
Stability of test compound:	The stability of dietary mixtures containing RP30228 was verified on a regular basis (see B.3 below). The homogeneity of dietary mixtures containing RP30228 was verified before the start of the study (250 and 13000 ppm) and after 4 weeks (815 and 13000 ppm).

2. Vehicle and/or positive control: Rodent diet

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Sex:	Male and female
Age:	6 weeks at the beginning of the treatment period
Mean weight at dosing:	♂: 173 g, ♀ 171 g
Source:	Centre d'Elevage Charles River, Saint-Aubin-les-Elbeuf, France
Acclimation period:	7 days
Diet:	A04 C fine ground diet (U.A.R., Villemoisson-sur-Orge, France), ad libitum
Water:	Tap water filtered using a 0.22 micron filter, ad libitum
Housing:	Pair housing (2 animals of the same sex and group/cage) in suspended wire-mesh cages (43.0 x 21.5 x 18.0 cm)
Environmental conditions:	
Temperature:	19 - 23 °C
Humidity:	30 - 70 %
Air changes:	13/hour
Photo period:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: (In life dates: 23-Jun-1992 (start of administration) to 24-Sep-1992 (necropsy))

2. Animal assignment and treatment:
RP30228 was administered to groups of 10 male and 10 female rats at dietary concentrations of 0, 815 (low dose), 3250 (intermediate dose) and 13000 ppm (top dose) for 13 weeks. The animals were assigned to the treatment groups by means of computer generated randomization lists based on body weights.

3. Test substance preparation and analysis:
For each group, the test substance was blended in a mortar with a small quantity of the diet. The required concentrations were then prepared by direct dilution of each premix with further quantities of untreated diet. Homogeneity was obtained by mixing for 10 minutes for a quantity sufficient for one week of treatment.

Before the beginning of the study, the homogeneity of dietary mixtures containing 250 and 13000 ppm RP30228 was checked. These concentrations were chosen as they were considered to represent the lowest and highest anticipated concentrations for use in the study. From each preparation, samples were taken at 3 different levels (top, middle and bottom) and analysed in duplicate. Due to a change of mixer from week 3 of treatment, the homogeneity of dietary mixtures containing 815 and 13000 ppm RP30228 was reverified on week 4. Before the beginning of the study, the stability of dietary mixtures containing 250 and 13000 ppm RP30228 was checked. Each preparation was kept either in closed bags and sampled the day of preparation and after 4, 7 and 14 days storage at room temperature or in open feeders and sampled the day of preparation and after 4, 7 and 10 days storage under animal room conditions. Each sample was analysed in duplicate. On weeks 1, 4, 8 and 12 of the study, each dietary mixture (control group included) was checked for the achieved concentration of the test substance (analysed in duplicate).

Relative standard deviations of the homogeneity samples in the range of 1.9 to 2.2% indicate the homogenous distribution of RP30228 in the diet preparations. The actual (mean) average test-substance concentrations were in the range of 96 to 106% of the nominal concentrations

4. Statistics:

The following sequence was used for the statistical analysis of body weight, food consumption, haematology, blood biochemistry, urinalysis and organ weight data:
The normality of the distribution of the values in each group was checked by Kolmogorov-Smirnov's test. If the distribution was normal, the homogeneity of variances between the groups was assessed by Bartlett's test (more than 2 groups) or Fisher's test (2 groups). If no significant heterogeneity of the variances was established, the comparison between treated and control groups was performed by Dunnett's test. If the variances were heterogeneous, the comparison between treated and control groups was performed by Dunn's test (more than 2 groups) or by Mann Whitney's test (2 groups). If the distribution of values in the groups was not normal, the analysis was repeated after logarithmic transformation of the values (except for organ weights). If this logarithmic transformation failed to normalise the distribution of the values, comparison of treated and control groups was performed by Dunn's test using original values.

C. Methods

1. Observations:

Clinical signs were observed for each animal, at least once a day, at the same approximate daily time. The animals were checked at least twice a day for possible mortality or signs of mordibity, including weekends and public Holidays.

2. Body weight:

Body weight was recorded for each animal, once before allocation into groups, on the first day of treatment, then once a week until the end of the study.

3. Food consumption, food efficiency and compound intake:

The quantity of food consumed by the animals was recorded once a week, over a 7-day period, throughout the treatment period. Food intake per animal and per day was calculated using the amount of food given and left in each food-hopper.

The achieved dosages of the test substance (in terms of mg/kg/day) was calculated on a weekly basis for each sex and each treated group using body weight and food consumption means and the nominal concentrations of the test substance in the diet:

$$\text{Achieveddosage}(\text{mg}/\text{kg}/\text{day}) = \frac{\text{Meanfoodconsumption}(\text{g}/\text{animal}/\text{day})}{\text{Meanbodyweight}(\text{g})} \text{ x Nominalconcentration}(\text{ppm})$$

Efficiency of food utilization was estimated by calculation of food ratios. Food conversion ratios were calculated on a weekly basis for each sex and each group, using body weight and food consumption means:

$$\text{Foodconsumptionratio} = \frac{\text{Meanfoodconsumption(g/animal/week)}}{\text{Meanbodyweightgain(g/animal/week)}}$$

4. Water consumption:

Not investigated.

5. Ophthalmoscopy:

Ophthalmological examinations were performed using an indirect ophthalmoscope on all animals of each sex and group, before the beginning of treatment period and on all animals of each sex of the control and high dose groups on week 13. Prior to examination, the pupils of all animals were dilated using an ophthalmic solution (Mydriaticum®, Merck Sharp, Dohme-Chibret, 75008 Paris, France), after examination of visual reflexes (retinal and corneal).

6. Functional observation battery (FOB):

Not performed.

7. Motor activity measurement:

Not performed.

8. Hematology and clinical chemistry:
Blood samples were taken from the orbital sinus of the overnight fasted animals under light ether anaesthesia. The samples were collected into tubes containing the appropriate anticoagulant.

The following hematological and clinical chemistry parameters were determined for all animals on week 13:

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time (Hepato Quick)
✓	Hemoglobin (Hb)	✓	Neutrophils (differential)	✓	Thrombocyte count
✓	Mean cell volume (MCV)	✓	Eosinophils (differential)		Activated partial thromboplastin time (APPT)
✓	Mean cell hemoglobin (MCH)	✓	Basophils (differential)	✓	Fibrinogen
✓	Mean cell Hb. conc. (MCHC)	✓	Lymphocytes (differential)		
✓	Packed cell volume (PCV)	✓	Monocytes (differential)		
Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium	✓	Albumin/globulin ratio	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Bilirubin (total)	✓	Aspartate aminotransferase (AST)
✓	Phosphorus (inorganic)	✓	Cholesterol	✓	Alkaline phosphatase (ALP)
✓	Potassium	✓	Creatinine		
✓	Sodium	✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

9. Urinalysis:
For urine collection, the animals were deprived of food and placed in metabolism cages. The urine was collected into a tube containing thymol crystals during an overnight period of about 22 hours.

The following parameters were determined for all animals on week 13:

Urinalysis					
	Quantitative parameters:		*Semiquantitative parameters*		
✓	Urine volume	✓	Bilirubin	✓	Nitrites
✓	Specific gravity	✓	Blood	✓	Protein
		✓	Appearance and color	✓	pH-value
		✓	Glucose	✓	Urobilirubin
		✓	Ketones	✓	Cytology (microscopical exam.)

10. Sacrifice and pathology:

On completion of the treatment period, after about 18 hours fasting, all animals were asphyxiated by carbon dioxide and sacrificed by exsanguination. The animals were weighed before necropsy. The following organs were sampled, weighed and examined histopathologically:

Pathology:

The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups). Paired organs were weighed separately. In all animals, all the macroscopic lesions and the tissues were preserved in 10% buffered formalin (except for the eyes and pituitary gland which were fixed in formolsublimate).

C	W	H		C	W	H		C	W	H	
✓	✓	✓	adrenals	✓	✓	✓	kidneys	✓		✓	seminal vesicles*
✓		✓	aorta	✓	✓	✓	liver	✓		✓	skin*
✓		✓	bone marrow[§]	✓		✓	lungs with bronchi	✓		✓	spinal cord (3 levels)[@]
✓		✓	brain (incl. medulla)/pons, cerebellar and cerebral cortex	✓		✓	lymph nodes[#]	✓	✓	✓	spleen
✓		✓	caecum	✓		✓	mammary gland	✓		✓	sternum w. marrow
✓		✓	colon	✓		✓	muscle, skeletal	✓		✓	stomach
✓		✓	duodenum	✓	✓	✓	ovaries	✓	✓	✓	testes
✓		✓	epididymides	✓		✓	pancreas	✓	✓	✓	thymus
✓		✓	esophagus	✓		✓	pituitary	✓		✓	thyroid/parathyroid
✓		✓	eyes	✓		✓	prostate*	✓		✓	tongue*
✓		✓	gross lesions	✓		✓	rectum	✓		✓	trachea
✓		✓	Harderian gland	✓		✓	salivary glands[$]	✓		✓	urinary bladder
✓	✓	✓	heart	✓		✓	sciatic nerve	✓		✓	uterus (horns, cervix)
✓		✓	ileum					✓		✓	vagina*
✓		✓	jejunum						✓	✓	body (anesthetized animals)

[§] from femur with articulation, [@] cervical, thoracic, lumbar; [#]mandibular and mesenteric, [$] (sublingual and submaxillary)

The tissues marked by * were preserved in fixative for possible future microscopic examination. All tissues required for microscopic examination were embedded in paraplast, sectioned at approximately 4 microns in thickness and stained with hemalum-eosin. Microscopic examination was performed on:

- all macroscopic lesions and tissues listed above (except those marked by *) in animals of the high dose and control groups sacrificed at the end of the treatment period.
- all macroscopic lesions and lungs, liver and kidneys of all the animals of the low and intermediate dose groups.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

See Section B.3 above.

B. OBSERVATIONS

1. Clinical signs of toxicity

No treatment-related clinical signs were observed throughout the study.

2. Mortality

No mortalities were observed during the course of the study.

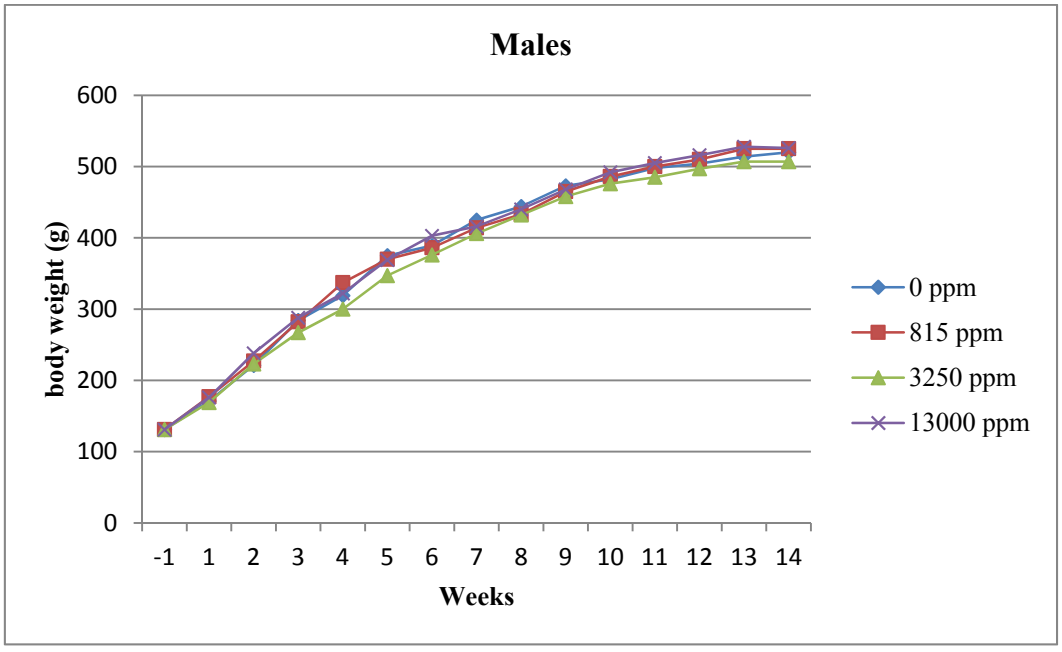
3. Ophthalmoscopy

No abnormalities were observed at the examinations performed before the beginning of the treatment and on week 13.

C. BODY WEIGHT AND BODY WEIGHT GAIN

The body weight gain of all treated males was similar to that of controls. A lower body weight gain was observed in all treated females. It was considered to be of no toxicological importance at 815 and 3250 ppm as it was minor (difference of body weight -9 and -7% respectively at the end of the treatment period) and due to the absence of a dose relationship. At the top dose, it was considered to be a slight adverse effect as it was more marked (difference of body weight -12% on week 14; see Figure 5.8.1-1, Table 5.8.1-11).

Figure 5.8.1-1: Body weight development of rats administered RP30228 for 13 weeks



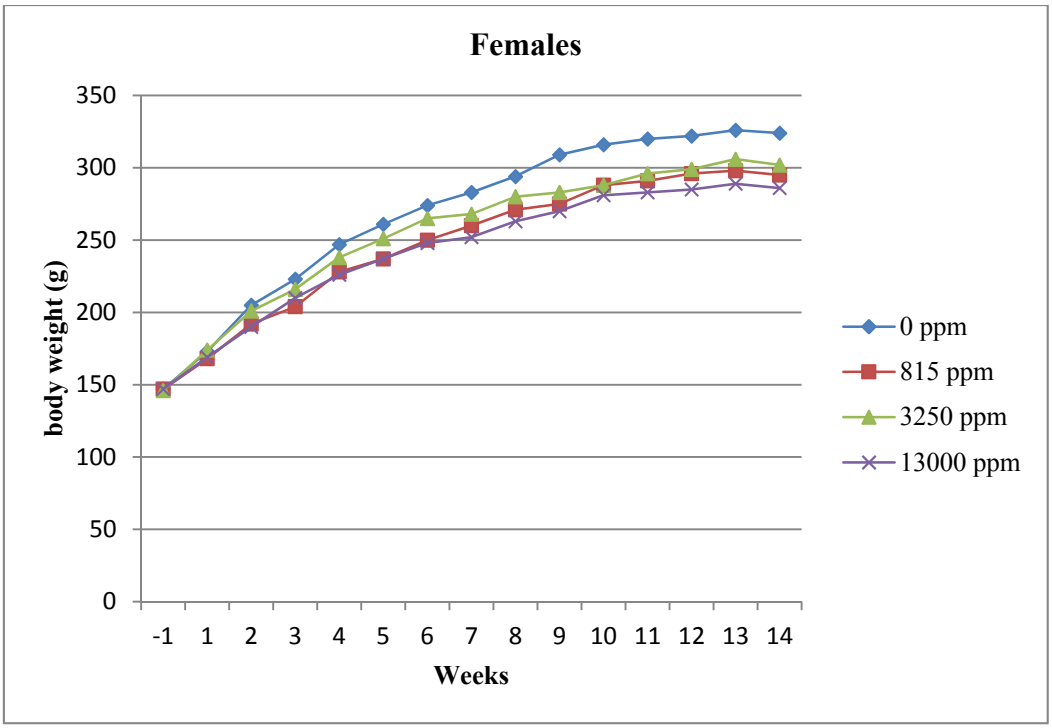


Table 5.8.1-11: Mean body weight of rats administered RP30228 F for 13 weeks

	Males				Females			
Dose level [ppm]	0	815	3250	13000	0	815	3250	13000
Body weight [g]								
- Week 1	131	131	131	131	147	147	146	147
- Week 4	319	337	300	322	247	228	238	226
- Week 8	444	433	432	440	294	271*	280	**263****
- Week 14	520	525	507	526	324	295*	302	**286****
Overall body weight gain [g] (weeks 1 to 14)	349	349	338	351	152	127	129	117

* $p < 0.05$; ** $p < 0.01$

D. FOOD CONSUMPTION AND COMPOUND INTAKE

The mean food consumption of males and females in all treated groups was similar to that of respective controls. The mean achieved dosages (in mg/kg/day) of males and females in treated groups is shown below, together with the overall range between week 1 and week 13 (see Table 5.8.1-12). The mean daily test substance intake was calculated to be 58, 234 and 954 mg/kg bw/day in males and 64, 252 and 1025 mg/kg bw/day in females at dietary dose levels of 815, 3250 and 1300 ppm, respectively.

Table 5.8.1-12: Mean achieved dosages between week 1 and 13

	Males			Females		
Dose level [ppm]	815	3250	13000	815	3250	13000
Week of treatment						
1	87	380	1513	79	353	1307
13	40	161	641	51	192	790
Mean	58	234	954	64	252	1025

Overall, the efficiency with which food was utilised by males in all treated groups was similar than that of controls.

In females, the efficiency of food utilization in treated groups was slightly lower than that of controls, reflecting those differences observed in body weight gain (see **Table 5.8.1-13**).

Table 5.8.1-13: Efficiency of food utilization (grams of food/unit of bw gain)

	Males				Females			
Dose level [ppm]	0	815	3250	13000	0	815	3250	13000
Weeks of treatment								
- Week 1-4	3.7	3.7	3.8	3.9	6.4	7.4	7.1	7.7
- Week 5-8	7.9	8.0	7.0	8.2	12.7	14.6	17.9	16.5
- Week 9-13	20.5	16.4	19.7	17.3	48.6	36.0	36.8	44.1
- Week 1-13	7.1	7.1	7.2	7.3	12.6	14.0	14.2	15.2

E. BLOOD ANALYSIS

1. Hematological findings

On week 13, the following statistically significant differences from control were noted:
- a slightly lower mean leucocyte count in males given 13000 ppm (7.8 vs. 9.5 G/L), due to a slightly lower neutrophil, lymphocyte and monocyte count
- a higher mean cell haemoglobin in males given 3250 ppm (17.7 vs. 16.9 pg)
- a slightly higher prothrombin time in females given 3250 and 13000 ppm (+19%)
- a slightly lower activated partial thromboplastin time in males given 13000 ppm (-20%)
- a slightly lower fibrinogen level in females given 3250 ppm and in animals of both sexes given 13000 ppm (-11% to -22%)

However, these differences from control were not dose-related and/or minor and the individual values were within or close to the normal range of the laboratories background data (not given in study report). Therefore, these findings were considered to be non-adverse. No abnormalities were noted among the other haematological parameters.

2. Clinical chemistry findings

The slight, but statistically significant difference from control noted among the blood biochemical parameters on week 13 (namely: sodium, potassium, chloride, glucose, creatinine) were minor, sporadic and not dose-related, and the individual values were within or close to the normal range of the historical background data (not given in study report). Therefore, these findings were considered to be non-adverse.

3. Urinalysis

Bilirubin was found in the urine of 2 out of 10 males given 13000 ppm. However, considering the low incidence of this finding, and in the absence of abnormalities in parameters related to kidney function, a relationship to the treatment was considered unlikely.

H. NECROPSY

1. Organ weight

A slight, but statistically significant increase in mean relative liver weight was noted in females given 815 ppm (+10%) and in males given 13000 ppm (+20%) when compared to respective values of controls. A statistically significant increase (+7%) in mean relative heart weight was also noted in females given 13000 ppm, when compared to controls. However, in the absence of relevant microscopic findings noted in the liver and heart in these groups, and in absence of a dose-relationship for the differences in liver weight, these differences from controls were considered to be of no toxicological importance. In male animals of the 815 ppm dose group a slight decrease in relative testes weight was observed (0.682 vs. 0.773 in controls; not statistically significant).

2. Gross and histopathology

Few macroscopic findings noted were those which are commonly recorded as spontaneous in laboratory rats of this strain.
With regard to the above described mean relative testes weight decrease observed in males at 815 ppm its worth mentioning that at gross pathology two of the males of this dose showed size-reduced testes, one of them with soft testes and the other one with additionally size-reduced epididymides. Histopathology of both males of this group revealed inhibited spermatogenesis (unilateral, grade 3) and degenerated seminiferous tubules (unilateral grade 3). Spermatic granuloma and oligospermia (unilateral, grade 4) in the epididymides was in addition observed in one of these males. However, as only 2/10 males were affected and no effects were observed on testes weights, gross- and histopathology of the mid and high dose males, the effects of the low dose were considered not to be treatment-related.
Further microscopic findings noted were those which are commonly recorded in untreated laboratory rats of this strain and age. Furthermore, their incidence, severity and morphological characteristics were similar between treated and control groups. Accordingly, they were considered to bear no relationship to treatment.

III. CONCLUSIONS

The no observed adverse effect level (NOAEL) under the conditions of the present study was determined to be 3250 ppm, which is equivalent to about 234 mg/kg bw/d in males and 252 mg/kg bw/d in females based on a lower body weight gain observed in females given 13000 ppm.

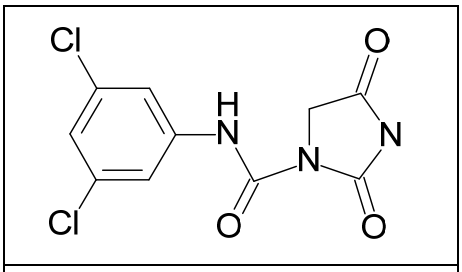
Assessment of subchronic toxicity of RP30228

RP30228 is of lesser subchronic toxicity than parent iprodione.

Toxicological evaluation of metabolite RP30228

QSAR analysis predicted a limited alert for genotoxicity *in vitro* with *in silico* metabolic activation which is considered of no relevance. Similar alerts were identified for iprodione and RP25040, which were both negative in the Ames test. Comparable acute toxicity after oral application in mice and rats to parent iprodione was observed and no potential for eye- or dermal irritation was seen. By weight of evidence RP30228 is not considered to be genotoxic. Moreover, RP30228 is considered as being of lesser toxicity after subchronic exposure than parent iprodione. A mechanistic study, not previously reported, indicated that RP30228 does not interact with the androgen receptor (BASF DocID R014678). RP30228 and RP36112 are placed into a common assessment group (1A), based on close structural similarity and participation of both metabolites in the same metabolic pathways. **In conclusion RP30228, assessed in a common metabolite group 1A with RP36112, is considered to be of lesser toxicity than parent iprodione. Therefore the human health based reference values for iprodione also apply to RP30228.**

Metabolite RP36112 Reg.No. 5079623



RP36112; Reg.No.5079623

Metabolite RP36112 is a structural isomer of iprodione and the de-alkylated metabolite of RP30228 determined in plant, livestock and rat. Based on close structural similarity to RP30228 and participation of both metabolites in the same metabolic pathways in plant, livestock and rat, these metabolites are placed into a common assessment group (1A), as agreed with the RMS. As there might be potential human consumer exposure via the food chain this metabolite was considered for toxicological relevance assessment.

Structural Alert for RP36112

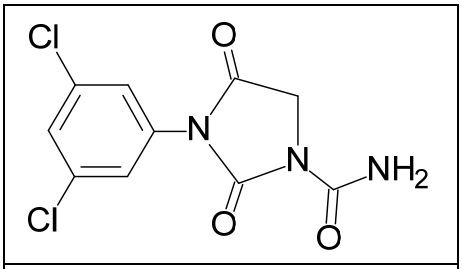
OASIS-Times gave a negative prediction of parent metabolite RP36112 genotoxicity but an in domain positive prediction after *in silico* metabolism for a DNA-reactive C-nitroso compound. This hypothetical occurring mutagenic compound potentially emerges after hydrolysis of the amide-group positioned between the dichlorophenyl- and dioxoimidazolidine-ring, giving rise to 3,5-dichloroanline and subsequently to the predicted mutagen. However, total predictivity was out of domain mutagen. Similar predictions for hypothetical occurring metabolites with C-nitroso compounds are proposed for 3,5-dichloroaniline, M610F007 and RP44247. 3,5-dinitroaniline as well as M610F007 and RP44247 were tested in Ames, including metabolic activation and were negative. Therefore, it was clearly demonstrated that the predicted metabolite is not relevant.
CAESAR predicted RP36112 as out of domain mutagen based on potential formation of metabolite CAS 330-54-1 (predicted DNA-reactive urea-derivative (NC=ON)). However, the underlying database for this prediction is poor, as the substance is clearly outside the applicability domain (global AD index 0; concordance 0). SarPy predicted RP36112 as non-mutagen potentially out of the applicability domain (global AD index 0.878; concordance 1).

In conclusion in two of the structure activity evaluation tools employed there was a limited alert for genotoxicity *in vitro* after *in silico* metabolic activation which is considered of low relevance based on similar predictions for 3,5-dichloroaniline, M610F007 and RP44247 for which *in vitro* genotoxicity data indicate non-mutagenicity. Overall there is no evidence that RP36112 is of genotoxic concern

Toxicological evaluation of metabolite RP36112

QSAR analysis predicted a limited alert for genotoxicity *in vitro* with *in silico* metabolic activation which is considered of no relevance as there is no concordance between QSAR models and the predicted metabolite was also identified for 3,5-dinitroaniline, M610F007 and RP44247, which were negative in the respective Ames test. RP36112 is structurally closely related to RP30228 for which acute toxicity, genotoxicity as well as repeated dose toxicity data is available leading to the conclusion that RP36112 is of comparable acute toxicit as parent, not genotoxic and of lesser subchronic toxicity than parent iprodione. Previously, a non-guideline mechanistic study *in vitro* conducted side-by-side with parent iprodione was performed and reported in the original dossier from 1997 (BASF DocID C029154), indicating that RP36112 may inhibit testosterone secretion in porcine Leydig cells at comparable concentrations to parent iprodione. Another mechanistic study, not previously reported, indicated that RP36112 does not interact with the androgen receptor (BASF DocID R014678). RP36112 and RP30228 are placed into a common assessment group (1A), based on close structural similarity and participation of both metabolites in the same metabolic pathways. **In conclusion RP36112, assessed in a common metabolite group 1A with RP30228, is considered to be of lesser toxicity than parent iprodione. Therefore the human health based reference values for iprodione also apply to RP36112.**

Metabolite RP32490 Reg.No. 5079628



RP32490; Reg.No.5079628

RP32490 is the de-alkylated metabolite of parent iprodione and determined in plant, livestock and rat. The determined level in rat is high (see chapter MCA 5.1), considering it a major metabolite and its toxicological properties covered by parent iprodione. Based on close structural similarity to RP25040 and participation of both metabolites in the same metabolic pathways in plant and rat, these metabolites are placed into a common assessment group (1B), as agreed with the RMS. As there might be potential human consumer exposure via the food chain this metabolite was considered for toxicological relevance assessment.

Structural Alert for RP32490

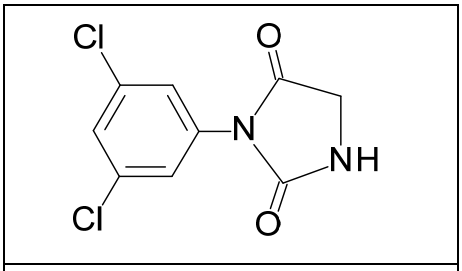
OASIS-Times gave a negative prediction of parent metabolite RP32490 genotoxicity. Moreover, total predictivity was out of domain negative.
CAESAR and SarPy predicted RP32490 as non-mutagen potentially out of the applicability domain.

In conclusion in none of the structure activity evaluation tools employed there was an alert for genotoxicity *in vitro* neither for parent nor after *in silico* metabolic activation.

Toxicological evaluation of metabolite RP32490

No relevant structural alerts were identified. RP32490 is considered a major rat metabolite (see chapter MAC 5.1) and the toxicological properties covered by parent iprodione. Previously, a non-guideline mechanistic study *in vitro* conducted side-by-side with parent iprodione was performed and reported in the original dossier from 1997 (BASF DocID C029154), indicating that RP32490 does not affect testosterone secretion in porcine Leydig cells. Another mechanistic study, not previously reported, indicated that RP32490 does not interact with the androgen receptor (BASF DocID R014678). RP32490 and RP25040 are placed into a common assessment group (1B), based on close structural similarity and participation of both metabolites in the same metabolic pathways. **In conclusion RP32490, assessed in a common metabolite group 1B with RP25040, is considered to be a major rat metabolite and the toxicological properties covered by parent iprodione. Therefore the human health based reference values for iprodione also apply to RP32490.**

Metabolite RP25040 Reg.No. 207099



RP25040; Reg.No.207099

RP25040 is the de-alkylated and de-amideated metabolite of iprodione and determined in plant, rat and soil. Based on close structural similarity to RP32490 and participation of both metabolites in the same metabolic pathways in plant and rat, these metabolites are placed into a common assessment group (1B), as agreed with the RMS. As there might be potential human consumer exposure via the food chain this metabolite was considered for toxicological relevance assessment.

Structural Alert for RP25040

OASIS-Times gave a positive prediction of parent metabolite RP25040 genotoxicity for DNA-reactive urea derivatives (NC=OC) without *in silico* activation. However, total predictivity was out of domain negative. Similar predictions for DNA-reactive urea-derivatives are proposed for parent iprodione and metabolite RP30228 after *in silico* metabolic activation.
CAESAR and SarPy predicted RP25040 as non-mutagen potentially out of the applicability domain.

In conclusion in one of the structure activity evaluation tools employed there was a limited alert for genotoxicity *in vitro* which is considered of no relevance based on similar predictions for parent iprodione and metabolite RP30228 for which *in vitro* genotoxicity data indicate non-mutagenicity. Moreover, *in vitro* genotoxicity data in bacterial cells for RP25040 indicates non-mutagenicity (BASF DocID C043625).

Acute toxicity studies for RP25040

Note: the acute oral toxicity and local tolerance study reports with metabolites RP25040 and RP37176 contain additional confidential information on impurities. Therefore the reports are located in the confidential part. To maintain the confidentiality of the study reports, those ones are not given in the public part of the AIR3 dosser, but the relevant data are summarized here. (BASF DocID R014584 and C043657)

Executive Summary acute oral toxicity of RP25040 and RP37176 (BASF DocID R014584)

In an acute oral toxicity study RP 25040 and RP 37176 diluted in 0.5% methylcellulose 400 in distilled water were each administered to fasted female CD1 mice via gavage. For assessment the "up and down" procedure was used starting with a single dose of 500 mg test substance/kg bw and an observation for 24 hours for survival or death. If the animal survived, a second animal was given a higher dose, increased by a factor of 1.5 up to a maximum dose level of 1125 mg/kg bw. If it died, the second animal was given a lower dose, decreased by the same factor. This procedure was repeated with further animals until reversal of the initial outcome was obtained. Three further animals were treated, their doses were adjusted up or down following the above-mentioned procedure. The approximate LD_{50} was calculated as the geometric mean of the last five figures (by the method of BRUCE, 1985). However, if no mortality occurred at 1125 mg/kg bw, the approximate LD_{50} was considered to be greater than 1125 mg/kg. At 1125 mg/kg bw, if one animal died and one animal survived, the approximate LD_{50} was considered to be equal to 1125 mg/kg bw.
No mortalities were observed for RP 37176. One death was observed for RP 25040. Accordingly, the following oral LD_{50} values were determined:

RP 25040: Oral LD_{50}: 1125 mg/kg bw
RP 37176: Oral LD_{50} > 1125 mg/kg bw

No clinical signs were observed for RP 37176. Numerous clinical signs were observed for animals treated with RP 25040 of which the principal clinical signs comprised absent righting reflex, could touch, staggering step and bradypnea at almost all dose levels (for details see summary below "B. Clinical Observations"). Since body weights were determined only once prior to the test substance administration, no conclusion on body weight development is possible. Necropsy was not performed.

(BASF DocID R014584)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: RP 25040, RP 37176

Description:	no data
Batch (test material):	EA2046RF1 (RP 25040), EA2062RF2 (RP 37176)
Purity:	no data
Stability of test compound:	no data

2. Vehicle: 0.5% methylcellulose 400 in distilled water

3. Test animals:

Species:	Mice
Strain:	CD1
Sex:	female
Age:	no data
Weight at dosing (mean):	20 - 29 g
Source:	Charles River, St Aubin Les Elbeuf, France
Acclimation period:	no data
Diet:	Certified Rodent Pellet diet A04C (Usine d'Alimentation Rationnelle, Villemoisson-sur-Orge, Epinay-sur-Orge, France; ad libitum
Water:	Municipal water, softened and filtered; ad libitum
Housing:	Animals were housed individually in Makrolon cages with autoclaved soft-wood bedding.

Environmental conditions:

Temperature:	22 ± 2°C (continuous control and recording)
Humidity:	55 ± 15% (continuous control and recording)
Air changes/hour:	approx. 15
Photo period:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 01-Apr-1992 - 21-Apr-1992

2. Animal assignment and treatment:

Mice were fasted overnight, weighed and given a single intragastric dose of the test formulation at a dosage volume of 20 mL/kg body weight. A total of 3 females was used for RP37176 and five animals for RP 25040 used in the assessment using the "up and down" procedure. For both compound, the first animal was given a single dose of 500 mg/kg bw and was observed for 24 hours for survival or death. If it survived, a second animal was given a higher dose, increased by a factor of 1.5 up to a maximum of 1125 mg/kg bw. If it died, the second animal was given a lower dose, decreased by the same factor. This procedure was repeated with further animals until reversal of the initial outcome was obtained (death to survival or survival to death). Three further animals were treated, their dose levels were adjusted up or down following the above mentioned procedure. When one animal given 1125 mg/kg bw survived, no further animals were treated.
All animals were checked for clinical signs and mortality during the first hours after dosing. Thereafter, individual clinical signs were recorded for each animal at least once daily for 7 days. In addition, animals were checked for mortality and moribundity once daily. At the end of the observation period on day 8, all surviving animals were sacrificed by an intraperitoneal injection of barbiturate. No necropsy was performed.

2. Calculation of the approximate lethal dose:

For the test substances inducing no mortality at 1125 mg/kg bw the approximate lethal dose was considered to be greater than 1125 mg/kg bw. At 1125 mg/kg wb, if one animal died and one animal survived, the approximate LD_{50} was considered to be equal to 1125 mg/kg bw. For the other outcomes, the logarithm of the last four doses, along with the logarithm of the extrapolated fifth dose was calculated. The anti-logarithm of the mean of the five calculated figures gave the approximate lethal dose.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortalities were observed RP 37176 (see Table 5.8.1-14). One death was observed for RP 25040.

Table 5.8.1-14: Mortality of rats administered RP 25040 and RP 37176 by gavage

Test susbtance	Sex	Dose (mg/kg bw)*	Mortality / dose
RP 37176	Female	500, 750, 1125	- / - / -
RP 25040	Female	500, 750A, 1125A, 750B, 1125B	- / - / Day 3 / - / -

*: A and B are two different animals for the dose concerned.

B. CLINICAL OBSERVATIONS

No clinical signs were observed for RP 37176.

For RP 25040 clinical signs comprised bradypnea, dyspnea, apnea, tremors, hunched posture, prostration, staggering step, emaciation, reduced motor activity, ptosis, reduced lacrimation, exophthalmia, reaction to noise, absent palpebral reflex, no reaction to pain, piloerection, cold touch, absent righting reflex, soiled urogenital area, lacrimation and bradychardia. Animals of all dose levels were affected and the clinical signs were observed on days 1 to 3.

C. BODY WEIGHT

Body weight of all animals was recorded on Day 1 prior to dosing. Afterwards body weights were not determined.

D. NECROPSY

Necropsy was not performed.

III. CONCLUSION

Under the experimental conditions of this study the oral LD_{50} of RP 25040 was determined to be 1125. For RP 37176 the oral LD_{50} in rmice was determined to be higher than 1125 mg/kg bw.

Executive Summary of toxicity and local tolerance of RP25040 in rabbits (BASF DocID C043657)

In a primary dermal irritation study the skin irritation/corrosion potential of RP25040 (Batch: No. 12 (RP25040)) was tested. The shaved intact or abraded clipped skin of 6 New Zealand White rabbits was exposed to 0.5 g of the test-substance using discs of about 3 cm diameter (discs of aluminium lined with gauze) under which the respective compound as a paste was applied. Contact was maintained for 24 hours using adhesive tape and the trunk of the animal was covered with a bandage to prevent licking. Observation of local reactions was performed 24 hours after application, when the discs were removed, and 72 hours after application. Data on body weight development or necropsy were not given in the study report. No erythema or edema of intact or abraded skin was noted after 24 and 72 hours in the 6 rabbits treated with RP25040. Individual skin reading scores were not given in the study report. Based on the findings of this study RP25040 do not show a skin irritation potential.

(BASF DocID C043657)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	RP25040 Metabolite of iprodione
Description:	no data
Batch / Test substance:	No. 12 (RP25040)
Purity	no data
Stability of test compound:	no data
2. Vehicle:	The test substances were applied unchanged**.**

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Sex:	no data
Age:	no data
Weight at dosing (mean):	2.5 - 3.0 kg
Source:	no data
Acclimation period:	no data
Diet:	no data
Water:	no data
Housing:	no data
Environmental conditions:	
Temperature:	no data
Humidity:	no data
Air changes:	no data
Photo period:	no data

B. STUDY DESIGN AND METHODS

1. Study completion date: 22-Apr-1974

2. Animal assignment and treatment:

The potential of RP25040 to cause acute dermal irritation or corrosion was assessed by a single topical application of the unchanged test substance (moistened with water) to the shaved intact or abraded skin of six New Zealand White rabbits. For application, small discs of about 3 cm diameter (discs of aluminium lined with gauze) were used, under each of which 0.5 g of the compound as a paste was applied. Contact was maintained for 24 hours using adhesive tape and the trunk of the animal was covered with a bandage to prevent licking. Observation of local reactions was performed 24 hours after application (i.e. when the discs were removed) and 72 hours after application. Data on body weight development or necropsy were not given in the study report.

II. RESULTS AND DISCUSSION

No erythema or edema of intact or abraded skin was noted after 24 and 72 hours in the 6 rabbits treated with RP25040. Individual skin reading scores were not given in the study report.

III. CONCLUSION

Based on the findings of this study RP25040 does not show a skin irritation potential under the test conditions chosen.

Assessment of acute toxicity for RP25040

RP25040 displays an acute oral toxicity to mice of 1125 mg/kg bw/d (R014584) and does not show a skin irritation potential.

Genotoxicity studies for metabolite RP25040

Note: the study reports of genotoxicity assessment in bacterial cells (AMES test) with metabolites RP25040 and RP37176 contain additional confidential information on impurities. Therefore the reports are located in the confidential part. To maintain the confidentiality of the study reports, those ones are not given in the public part of the AIR3 dosser, but the relevant data are summarized here. (BASF DocID C043625)

Executive Summary

S. typhimurium strains TA98, TA100, TA1535 and TA1537 were exposed to RP 25040 and RP 37176 (Batch: see below; Purity: 99.7 and 99.9%, respectively) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in a plate incorporation experiment. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment. A preliminary cytotoxicity test versus several hundred bacteria of strain TA100 was carried out both in the presence and absence of a mammalian microsomal metabolic activation system to determine appropriate dose levels of the test substance to be used in the mutagenicity test. Concentrations ranging from 0.05 to 5000 µg/plate were used in the assays with and without metabolic activation, respectively. At least five concentrations per test substance were investigated. Precipitation and reduced background lawn was observed depending on the test conditions and strains mostly at higher concentrations in almost all of the experiments. With respect to all test substances, 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 substances RP 25040 and RP 37176 are not mutagenic in the Salmonella typhimurium reverse mutation assay under the experimental conditions of the study.

(BASF DocID C043625)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material RP 25040 (plant metabolite of iprodione)
Description: Powder, white
Lot/Batch #: EA2046RF1
Purity: 99.7%
Stability of test compound: no data
Solvent used: Dimethylsulfoxide (DMSO)

Test Material RP 37176 (plant metabolite of iprodione)
Description: Fluffy powder, white
Lot/Batch #: EA2062RF2
Purity: 99.9%
Stability of test compound: no data
Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:
Negative control: Not included into the study design.
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.

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
All strains	2-aminoanthracene (2-AA)	DMSO	2 µg/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	Sodium azide (NaN_3)	Ultrapure water	1 µg/plate
TA 1535			
TA 98	2-nitrofluorene (2-NF)	DMSO	1 µg/plate
TA 1537	9-aminoacridine (9-AA)	DMSO	50 µg/plate

3. Activation:

The 9000 g post-mitochondrial liver supernatant fraction (S9) was obtained from SD-OFA male rats treated with Aroclor 1254 (500 mg/kg, i.p.) 5 days prior to sacrifice. S9 was supplied by Iffa Credo (France). The metabolic activation system (S9 mix) was prepared immediately prior to use and contained the following components prepared in ultrapure water:

Component	Concentration
Sodium phosphate buffer (pH 7.4)	100 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

5. Test concentrations:

Plate incorporation assay:

For all tester strains indicated above, each mutagenicity test incorporated a range of at least 5 concentrations (3 plates/concentration) of the test substance which were separated by factors of 2-3 and with the highest test substance concentration being one or more of the following:
- 5000 µg/plate if no test substance precipitate or toxic effect were noted up to this level in the preliminary cytotoxicity test.
- a concentration producing a slight precipitate
- a concentration producing slight toxicity (as indicated by the thinning of the background lawn). In general this was the test substance concentration demonstrated to reduce the survival rate of strain TA100 to approximately 20 % in the preliminary cytotoxicity assay.

B. TEST PERFORMANCE:

1. Dates of experimental work: 08-Jul-1992 to 30-Nov-1992

2. Toxicity test:

A preliminary cytotoxicity test versus several hundred bacteria of strain TA100 was carried out both in the presence and absence of a mammalian microsomal metabolic activation system to determine appropriate dose levels of the test substance to be used in the mutagenicity test. The results of the cytotoxicity test were expressed as the percentage of surviving colonies at each test substance concentration in relation to the number of colonies in the negative-solvent control.

3. Plate incorporation assay:

For the mutagenicity test, minimal glucose agar plates were prepared containing 1.5% bacteriological grade agar and 2% glucose in 20 mL of Vogel Bonner medium E. For the cytotoxicity test, plates were prepared in the same manner except that, prior to pouring, agar was supplemented (1 mL per 100 mL of agar) with an aqueous solution containing 32 mM histidine and 0.3 mM biotin.
Top agar contained 0.6% bacteriological grade agar and 0.5% sodium chloride. Prior to use, this solution was supplemented (1 mL per 10 mL of agar) with an aqueous solution containing 0.5 mM histidine and 0.5 mM biotin. For the conduction of the cytotoxicity and mutagenicity tests, the top agar was distributed, 2 or 2.5 mL for assays with or without metabolic activation respectively, into culture tubes and maintained, in a molten state, at 45°C.
The following solutions were added in this order:
- 0.1 mL of a solution of the test substance or a solution of the positive control or the solvent alone.
- 0.1 mL of the bacterial cell culture (a one millionth dilution for the cytotoxicity test and undiluted for the mutagenicity test).
- 0.5 mL of S9 mix for tests with metabolic activation. This mixture was then distributed evenly over the surface of minimal glucose agar plates.
For the cytotoxicity test, plates were stored inverted for 36 - 60 h at 37°C. For the mutagenicity test, plates were stored inverted for 60 h at 37°C. Following the incubation period the number of histidine-independent colonies, in the case of the mutagenicity test, and histidine-dependent colonies, in the case of the cytotoxicity test, were counted. Plates in which there was evidence that the test substance has precipitated were recorded separately. The bacterial background lawn was checked visually on all plates used in the mutagenicity test.

3. Statistics:

No special statistical tests were performed.

4. Evaluation criteria:

A ratio "R" was also used to express the mutagenic effect at each test substance concentration. This was calculated as follows:

R = mean number of revertant colonies induced on plates of a given test substance concentration / mean number of revertant colonies spontaneously induced on plates of the negative-solvent control

No further details on evaluation were given in the study report.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Not performed.

B. TOXICITY AND MUTATION ASSAYS

RP 25040

RP 25040 was tested to the limits of cytotoxicity and/or solubility (2500 µg/plate in the absence of S9 mix and 5000 µg/plate in the presence of S9 mix) and did not induce any significant increases in the revertant numbers of any strain at any of the test substance concentrations studied. RP 25040 was considered to be non-mutagenic under the conditions of this assay (see Table 5.8.1-15)

Table 5.8.1-15: Bacterial gene mutation assay with RP 25040 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Solvent control	40	23	105	105	20	18	21	11
RP 25040								
100 µg/plate	37	17	109	102	15	18	13	11
250 µg/plate	37	22	97	88	17	19	18	10
500 µg/plate	36	29	95	82	16	16	12	7
1000 µg/plate	31	18	109	84	11	21	16	8
2500 µg/plate	34	22P	81	77P	14	20P	13	6P*
5000 µg/plate	UP	-	UP	-	UP	-	UP	-
Pos. control[§]	1337	307	2138	402	224	303	256	123

[§] = Compound and concentrations see Material and Methods (I.A.2.) above
P = precipitate
U = plate unreadable
* = inhibition of background lawn

RP 37176

RP 37176 was tested to the limits of cytotoxicity (10 µg/plate in the absence of S9 mix and 500 µg/plate in the presence of S9 mix) and did not induce any significant increases in the revertant numbers of any strain at any of the test substance concentrations studied. RP 37176 was considered to be non-mutagenic under the conditions of this assay (see Table 5.8.1-16).

Table 5.8.1-16: Bacterial gene mutation assay with RP 37176- Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Solvent control	40	30	114	106	23	23	22	15
RP 37176								
0.5 µg/plate	-	21	-	102	-	22	-	15
1 µg/plate	-	30	-	101	-	25	-	13
2.5 µg/plate	-	31	-	92	-	23	-	12
5 µg/plate	-	28	-	112	-	22	-	14*
10 µg/plate	-	32*	-	92*	-	23*	-	11*
25 µg/plate	39	-	119	-	20	-	20	-
50 µg/plate	41	-	110	-	21	-	30	-
100 µg/plate	36	-	112	-	20	-	23	-
250 µg/plate	32*	-	106*	-	17*	-	16*	-
500 µg/plate	41*	-	78*	-	15*	-	15*	-
Pos. control§	1157	255	1710	453	236	335	225	146

§ = Compound and concentrations see Material and Methods (I.A.2.) above
* = inhibition of background lawn

III. CONCLUSION

According to the results of the present study, the test substances RP 25040 and RP 37176 are not mutagenic in the Salmonella typhimurium reverse mutation assay under the experimental conditions chosen here.

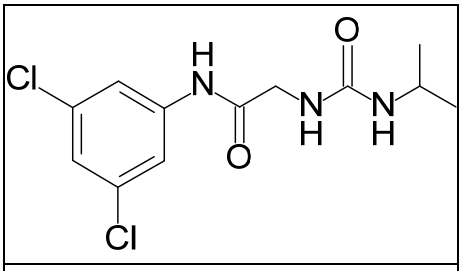
Assessment of genotoxicity for RP25040

RP25040 does not cause point mutations in bacterial cells

Toxicological evaluation of metabolite RP25040

QSAR analysis predicted a limited alert for genotoxicity which is considered non-relevant as no evidence for genotoxicity was observed *in vitro*. Acute toxicity in mice and rabbits indicates comparable toxicity to parent iprodione. Previously, a non-guideline mechanistic study *in vitro* conducted side-by-side with parent iprodione was performed and reported in the original dossier from 1997 (BASF DocID C029154), indicating that RP25040 does not affect testosterone secretion in porcine Leydig cells. Another mechanistic study, not previously reported, indicates that RP25040 may interact very weakly with the androgen receptor at concentration levels of no biological relevance (BASF DocID R014678). RP25040 and RP32490 are placed into a common assessment group (1B), based on close structural similarity and participation of both metabolites in the same metabolic pathways. **In conclusion, the toxicological properties of RP25040, assessed in a common metabolite group 1B with major rat metabolite RP32490, are considered covered by parent iprodione. Therefore the human health based reference values for iprodione also apply to RP25040.**

Metabolite RP37176 Reg.No. 5079612



RP37176; Reg.No.5079612

RP37176 is a dioxoimidazolidine-opened metabolite of parent iprodione determined after hydrolysis, only. No structural similarity to any other metabolite is apparrent; therefore RP37176 is placed into a single metabolite assessment group (2A) as agreed with RMS. As there might be potential human consumer exposure via the food chain this metabolite was considered for toxicological relevance assessment.

Structural Alert for RP37176

OASIS-Times gave a negative prediction of genotoxicity for parent metabolite RP37176 but an in domain positive prediction after *in silico* metabolism for a DNA-reactive hydroxyalamine. This hypothetical occurring mutagenic compound potentially emerges after hydrolysis of the dichlorophenyl-ring closest amide-group, giving rise to 3,5-dichloroanline and subsequently to a hydroxylamine. However, total predictivity was out of domain mutagen. Similar predictions for hypothetical occuring hydroxylamines or C-nitroso compounds, of which hydroxylamines are precursors of, are proposed for 3,5-dichloroaniline, M610F007, RP36221 and RP44247. As 3,5-dinitroaniline as well as M610F007, RP36221 and RP44247 were tested negative this alert has no relevance.
CAESAR predicted RP37176 as out of domain mutagen based on potential formation of metabolite CAS 330-54-1 (predicted DNA-reactive urea-derivative (NC=ON)). However, the underlying database for this prediction is poor, as the substance is clearly outside the applicability domain (global AD index 0; concordance 0). SarPy predicted RP37176 as non-mutagen potentially out of the applicability domain (global AD index 0.883; concordance 1).

In conclusion in two of the structure activity evaluation tools employed there was a limited alert for genotoxicity *in vitro* after *in silico* metabolic activation which is considered of no relevance based on similar predictions for 3,5-dichloroaniline, M610F007, RP36221 and RP44247 for which *in vitro* genotoxicity data indicate non-mutagenicity. Moreover, *in vitro* genotoxicity in bacterial cells for RP37176 indicates non-mutagenicity (BASF DocID C043625).

Acute studies for metabolite RP37176

Note: the acute oral toxicity study reports with metabolites RP37176 and RP25040 contain additional confidential information on impurities. Therefore the report is located in the confidential part. To maintain the confidentiality of the study reports, those ones are not given in the public part of the AIR3 dosser, but the relevant data are summarized in acute toxicity studies of metabolite RP25040; see above). (BASF DocID C043657**)**

Assessment of acute toxicity for RP 37176

For RP 37176 the oral LD_{50} in rmice was determined to be higher than 1125 mg/kg bw (BASF DocID R014584).

Genotoxicity studies for metabolite RP37176

Note: the study reports of genotoxicity assessment in bacterial cells (AMES test) with metabolites RP37176 and RP25040 contain additional confidential information on impurities. Therefore the report is located in the confidential part. To maintain the confidentiality of the study reports, those ones are not given in the public part of the AIR3 dosser, but the relevant data are summarized in genotoxicity studies for metabolite RP25040; see above (C043625).

In addition the following new study is submitted :

Report:	CA 5.8.1/6 Bohnenberger S., 2013a Reg.No. 5079612 (metabolite of BAS 610 F, Iprodione) - In vitro micronucleus test in Chinese hamster V79 cells 2013/1224040
Guidelines:	OECD 487 (2010), Commission Regulation EU No. 640/2012 of 06 July 2012 - B.49: In vitro Mammalian Cell Micronucleus Test
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

Reg. No. 5079612 (Batch L80-184; purity 99.7%) was tested in vitro for its potential to induce micronuclei in V79 cells of the Chinese hamster in the absence and presence of metabolic activation by S9 mix in two independent experiments. Concentrations of 2.8 to 1450 µg/mL were tested within an exposure period of 4 hours in a pre-test with and without metabolic activation. Since no cytotoxicity was observed, this pre-test was designated Experiment I. In addition, cells were continuously treated for 24 hours (Experiment II). The cells were prepared 24 hours after start of treatment with the test item. Vehicle (DMSO) and positive controls (without metabolic activation: Mitomycin C, Griseofulvin; with metabolic activation: Cyclophosphamide) were included to demonstrate the sensitivity of the test system. In each experimental group two parallel cultures were set up and at least 1000 cells per culture were scored for micronuclei. Precipitation of the test item in the culture medium was observed at the end of treatment at 45.3 µg/mL and above in Experiment I in the absence of S9 mix and in Experiment II in the absence and presence of S9 mix. In Experiment I in the presence of S9 mix precipitation was observed at 90.6 µg/mL and above. In Experiment I and II in the absence and presence of S9 mix no cytotoxicity was observed up to the highest applied concentration. No biologically relevant mutagenicity was observed in any test culture with and without S9 mix.

However, in Experiment I with metabolic activation an increase in micronucelated cells was observed at 90.6 µg/mL. Since the dose level exceeded the solubility of the test compound, causing precipitation to occur, and the value was not statistically significant the finding has to be regarded as biologically irrelevant. A single significant increase in cells with micronuclei at 11.3 µg/mL was observed in Experiment II without metabolic activation. In the same experiment in the presence of S9 mix, statistically significant increases in micronucleated cells were observed after treatment with 11.3, 22.7 and 45.3 µg/mL. These values did not display a dose-dependent increase in micronucleated cells and were clearly in the range of the laboratory historical control data and were therefore considered as biologically irrelevant. Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test. Based on the results of this study Reg. No. 5079612 is considered to not induce micronuclei in vitro in V79 cells in the presence or absence of metabolic activation.

(BASF DocID 2013/1224040)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Reg. No. 5079612 (Metabolite of BAS 610 F, iprodione)
Description:	Solid, beige
Lot/Batch #:	L80-184
Purity:	99.7%
Stability of test compound:	The stability of the test substance dissolved in the vehicle DMSO was analytically confirmed (for details [see 2014/1095067 Becker M.,Kamp H. 2014 b]).
Solvent used:	Dimethylsulfoxide (DMSO)
2. Control Materials:	
Negative control:	A negative control was not employed in this study
Solvent control:	DMSO
Positive controls, -S9:	Mitomycin C (MMC, 0.3 µg/mL, dissolved in deionised water)
	Griseofulvin (9.0 µg/mL, dissolved in DMSO)
Positive control, +S9:	Cyclophosphamide (CPA, 0.5 µg/mL dissolved in saline)

3. Activation:

S9 was produced from male Wistar rats. The rats were induced by intraperitoneal applications of 80 mg/kg bw phenobarbital and by oral administrations of 80 mg/kg bw β-naphthoflavone each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g. Aliquots of the supernatant were frozen and stored in ampoules at –80 °C. The S9 mix preparation was performed according to Ames et al. Briefly, an appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution (see below) to result in a final protein concentration of 0.75 mg/mL in the cultures. During the experiment the S9 mix was stored on ice.

The S9-mix was prepared immediately before use and had the following composition:

Component	*Concentration*
Sodium phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

4. Test organisms:

Chinese hamster V79 cells

This is a continuous cell line with a population doubling time of 13 hours.

5. Culture medium/conditions: About 5 x 10^5 cells/flask were seeded in 15 mL of MEM (minimal essential medium) containing Hank's salts, glutamine, Hepes (25 mM), penicillin/ streptomycin (100 U/mL/100 mg/mL) and 10 % (v/v) fetal bovine serum (FBS). Cell cultures were incubated at 37 °C in a humidified atmosphere with 1.5% carbon dioxide (98.5% air).

Exponentially growing stock cultures more than 50 % confluent were rinsed with Ca-Mg-free salt solution. Afterwards the cells were treated with trypsin-EDTA-solution at 37 °C for approx. 5 minutes. By adding complete culture medium including 10 % (v/v) FBS the enzymatic treatment was stopped and a single cell suspension was prepared. The cells were seeded into Quadriperm dishes, which contained microscopic slides. Into each chamber 1.0 x 10^5 – 1.5 x 10^5 cells were seeded with regard to the preparation time. In each experimental group two parallel cultures were set up.

6. Test concentrations:

a) Preliminary toxicity assay: 2.8 - 1450.0 µg/mL with and without metabolic activation

Since no cytotoxicity was observed, the pre-test was designated Experiment I (see Table 5.8.1-17).

b) Cytogenicity assay: 2.8 - 1450.0 µg/mL with and without metabolic activation (Experiment II)

Table 5.8.1-17: Doses applied in the Micronucleus Test in Chinese Hamster V79 Cells with Reg.No. 5079612 (Metabolite of BAS 610 F, iprodione)

Preparation interval	Exposure period	Exp.	Concentration in µg/mL									
			Without S9 mix									
24 hrs	4 hrs	I	2.8	5.7	**11.3**	**22.7**	**45.3^P**	90.6^P	181.3^P	362.5^P	725.0^P	**1450.0^P**
24 hrs	24 hrs	II	2.8	5.7	**11.3**	**22.7**	**45.3^P**	90.6^P	181.3^P	362.5^P	725.0^P	1450.0^P
			With S9 mix									
24 hrs	4 hrs	I	2.8	5.7	11.3	**22.7**	**45.3**	**90.6^P**	181.3^P	362.5^P	725.0^P	**1450.0^P**
24 hrs	4 hrs	II			**11.3**	**22.7**	**45.3^P**	90.6^P	181.3^P	362.5^P	725.0^P	1450.0^P

Evaluated experimental points are shown in bold characters

P Precipitation occurred at the end of treatment

B. TEST PERFORMANCE:

1. Dates of experimental work: 24-Apr-2013 - 23-May-2013

2. Preliminary cytotoxicity assay: With respect to the solubility of the test item, 1450.0 µg/mL of Reg. No. 5079612 was applied as top concentration for treatment of the cultures in a pre-test. Test item concentrations between 2.8 and 1450.0 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity.

3. Cytogenicity Assay:

Exposure period 4 hours: The culture medium of exponentially growing cell cultures was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL medium was added.

Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed twice 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. Then the cells were cultured in complete medium containing 10% (v/v) FBS for the remaining culture time of 20 hours.

Exposure period 24 hours: The culture medium of exponentially growing cell cultures was replaced with complete medium containing 10% (v/v) FBS including the test item without S9 mix. The medium was not changed until preparation of the cells. Concurrent solvent and positive controls were performed.

Preparations of cultures:

For the micronucleus analysis, 24 hours after the start of the exposure, the cells were treated on the slides in the chambers of the quadriperm dishes with deionised water for 1 to 1.5 min at 37 °C. Afterwards the cells were fixed twice with a solution containing 3 parts ethanol, 1 part acetic acid and 1.25 % (v/v) formaldehyde. After preparation the cells were stained with Giemsa and labelled with a computer-generated random code to prevent scorer bias.

Analysis of micronuclei and cytotoxicity:

Evaluation was performed manually using microscopes with 40x objectives. The micronuclei were stained in the same way as the main nucleus. The area of the micronucleus did not extend the third part of the area of the main nucleus. 1000 cells in two parallel cultures were scored for micronuclei, so that at least 2000 cells from clones with 2 - 8 cells were analysed per test group. The frequency of micronucleated cells was reported as % micronucleated cells.

Cytotoxicity was assessed via counting the number of clones consisting of 1 cell (c1), 2 cells (c2), 3 - 4 cells (c4), and 5 - 8 cells (c8) among the cells that were scored for the presence of micronuclei. These clusters represented the cells that have divided 1, 2, or 3 times within the experiment. From these data, a proliferation index (PI) was calculated (see formula below). Only those cultures were evaluated which showed a PI > 1.3, in order to guarantee for a sufficient cell proliferation during treatment and recovery.

$$PI = \frac{(c1 \times 1) + (c2 \times 2) + (c4 \times 3) + (c8 \times 4)}{(c1 + c2 + c4 + c8)}$$

PI: Proliferation index
cx: Number of clones with x cells (with x: 1, 2, 4, or 8)

4. Statistics:

Statistical significance at the five per cent level was evaluated by means of the Chi-square test. Evaluation was performed only for test groups showing a higher number of micronucleated cells than the respective solvent control group.

5. Evaluation criteria:

A test item was classified as mutagenic if:

- the number of micronucleated cells is not in the range of the historical 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.

A test item can be classified as non-mutagenic if:

- the number of micronucleated cells in all evaluated test groups is in the range of the historical control data and
- no statistically significant or concentration-related increase in the number of micronucleated cells is observed in comparison to the respective solvent control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

The stability of the test substance under storage conditions throughout the study period was guaranteed by the sponsor. (see Annex III of the final study report). The stability of the test substance dissolved in the vehicle DMSO was analytically confirmed (for details [see 2014/1095067 Becker M.,Kamp H. 2014 b]).

B. PRELIMINARY CYTOTOXICITY ASSAY:

No cytotoxicity was observed in the pre-test. Since the cultures fulfilled the requirements for cytogenetic evaluation, the preliminary test was designated Experiment I (see below).

C. CYTOGENICITY ASSAYS:

Two independent experiments were performed. In Experiment I the exposure period was 4 hours with and without metabolic activation. In Experiment II the exposure period was 24 hours without S9 mix and 4 hours with metabolic activation. The cells were prepared 24 hours after start of treatment with the test item. Visible precipitation of the test item in the culture medium was observed microscopically at the end of treatment at 45.3 µg/mL and above in Experiment I in the absence of S9 mix and in Experiment II in the absence and presence of S9 mix. In Experiment I in the presence of S9 mix precipitation was observed microscopically at the end of treatment at 90.6 µg/mL and above (see Table 5.8.1-17). No relevant influence on osmolarity or pH value was observed. In Experiment I and II in the absence and presence of S9 mix no cytotoxicity was observed up to the highest applied concentration. No mutagenicity was observed with and without S9 mix (see Table 5.8.1-18). The rates of micronucleated cells after treatment with the test item in Experiment I without S9 mix and in Experiment II with and without S9 mix (0.40 - 1.00%) were within the rates of the solvent control values (0.30 - 1.10%) and within the range of the laboratory historical solvent control data. In Experiment I in the presence of S9 mix one single increase in micronucleated cells (1.75%) slightly above the range of the laboratory historical control data (0.05 - 1.70% micronucleated cells) was observed after treatment with 90.6 µg/mL. Since the dose level exceeded the solubility of the test compound, causing precipitation to occur, and the value is not statistically significant the finding has to be regarded as biologically irrelevant. In Experiment II in the absence of S9 mix one single statistically significant increase in micronucleated cells (0.90%) was observed after treatment with 11.3 µg/mL. In the presence of S9 mix statistically significant increases in micronucleated cells were observed after treatment with 11.3, 22.7 and 45.3 µg/mL (0.75, 0.90 and 0.80%). These values did not display a dose-dependent increase in micronucleated cells and were clearly in the range of the laboratory historical control data (without S9 mix: 0.05 – 1.50%; with S9 mix: 0.05 - 1.70% micronucleated cells) and are therefore considered as biologically irrelevant. Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test.

Table 5.8.1-18: Summary of results of micronucleus test with Reg. No. 5079612 (Metabolite of BAS 610 F, iprodione)

Exp.	Preparation interval	Test item concentration [µg/mL]	Proliferation index	Micronucleated Cells* [%]
Exposure period 4 hrs without S9 mix				
I	24 hrs	Solvent control[1]	2.89	1.10
		Positive control[2]	2.23	**11.20[s]**
		11.3	2.88	0.85
		22.7	2.94	1.00
		45.3[P]	2.87	0.50
		1450.0[P]	2.87	n.e.
Exposure period 24 hrs without S9 mix				
II	24 hrs	Solvent control[1]	3.26	0.30
		Positive control[3]	2.93	**16.05[s]**
		11.3	3.20	**0.90[s]**
		22.7	3.26	0.55
		45.3[P]	3.11	0.40
Exposure period 4 hrs with S9 mix				
I	24 hrs	Solvent control[1]	1.99	1.30
		Positive control[4]	1.48	**9.50[s]**
		22.7	2.39	1.50
		45.3	2.21	1.20
		90.6[P]	2.20	1.75
		1450.0[P]	2.21	n.e.
II	24 hrs	Solvent control[1]	2.20	0.30
		Positive control[4]	1.81	**8.50[s]**
		11.3	2.24	**0.75[s]**
		22.7	2.18	**0.90[s]**
		45.3[P]	2.18	**0.80[s]**

* The total number of micronucleated cells was determined in a sample of 2000 cells
n.e. Not evaluated since the highest applied concentration is not cytotoxic
[s] Number of micronucleated cells statistically significantly higher than corresponding control values
[P] Precipitation occurred microscopically at the end of treatment
[1] DMSO 0.5 % (v/v)
[2] Mitomycin C 0.3 µg/mL
[3] Griseofulvin 9.0 µg/mL
[4] CPA 10.0 µg/mL

III. CONCLUSIONS

Based on the results of the study it is concluded that Reg. No. 5079612 does not induce micronuclei in V79 cells in the presence or absence of metabolic activation

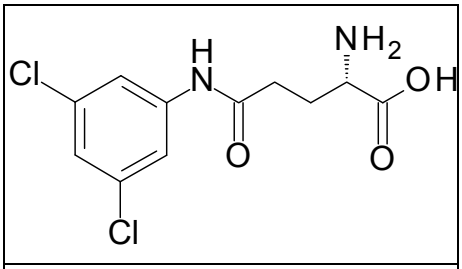
Assessment of genotoxicity for RP37176

RP37176 does not cause point mutations in bacterial cells (BASF DocID C043625) nor does it induce micronuclei *in vitro*. Overall there is no evidence that RP37176 is genotoxic *in vitro*.

Toxicological evaluation of metabolite RP37176

QSAR analysis predicted a limited alert for genotoxicity *in vitro* with *in silico* metabolic activation which is considered of no relevance. Acute toxicity in mice revealed comparable oral toxicity to parent iprodione (BASF DocID R014584). There is no evidence that RP37176 is genotoxic *in vitro*. No structural similarity to any other metabolite is apparrent; therefore RP37176 is placed into a single metabolite assessment group (2A). **In conclusion, RP37176 is considered to be of no genotoxicological concern. Therefore the human health based reference value of 1.5µg/kg bw/d according to the TTC concept for compounds of Cramer Class III can be applied.**

Metabolite M610F007 Reg.No. 5916256



M610F007; Reg.No.5916256

M610F007 is a conjugate of 3,5-dichloroaniline and naturally occurring glutamic acid determined in carrot root, only. No structural similarity to any other metabolite is apparent; therefore M610F007 is placed into a single metabolite assessment group (2B) as agreed with RMS. As there might be potential human consumer exposure via the food chain this metabolite was considered for toxicological relevance assessment.

Structural Alert for M610F007

OASIS-Times gave a negative prediction of parent metabolite M610F007 genotoxicity but an in domain positive prediction after *in silico* metabolism for a DNA-reactive C-nitroso compound. This hypothetical occurring mutagenic compound potentially emerges after hydrolysis of the dichlorophenyl-ring closest amide-group, giving rise to 3,5-dichloroanline and subsequently to the predicted mutagen. However, total predictivity was out of domain mutagen. Similar predictions for hypothetical occurring metabolites with C-nitroso compounds are proposed for 3,5-dichloroaniline and RP44247. 3,5-dichloroaniline as well as RP44247 were tested negative in Ames clearly demonstrating that this alert is not relevant.
CAESAR and SarPy predicted M610F007 as in domain non-mutagen.

In conclusion in one of the structure activity evaluation tools employed there was a limited alert for genotoxicity *in vitro* after *in silico* metabolic activation which is considered of no relevance based on similar predictions for 3,5-dichloroaniline and RP44247 for which *in vitro* genotoxicity data indicate non-mutagenicity. Moreover, in vitro mutagenicity data for M610F007 indicate non-mutagenicity (BASF DocID 2014/1082362).

Genotoxicity studies for metabolite M610F007

Report:	CA 5.8.1/7 Woitkowiak C., 2014a Reg.No. 5916256 (metabolite of BAS 610 F, Iprodione) - Salmonella typhymurium / Escherichia coli - Reverse mutation assay 2014/1082362
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. 5916256 (Metabolite of BAS 610 F, iprodione); Batch: L80-176; Purity: 99.8%) using DMSO as a solvent in the presence and absence of metabolic activation in a plate incorporation assay and a pre-incubation experiment. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment. In the plate incorporation assay as well as in the pre-incubation assay, Reg.No. 5916256 was tested at concentrations of 33, 100, 333, 1000, 2500 and 5000 µg/plate. A bacteriotoxic effect was occasionally observed depending on the strain and test conditions from about 333 µg/plate onwards. 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. 5916256 is not considered mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2014/1082362)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Reg.No. 5916256 (Metabolite of BAS 610 F, iprodione)

Description: Solid, white

Lot/Batch #: L80-176

Purity: 99.8% (tolerance +/- 1.0%)

Stability of test compound: The stability of the test substance under storage conditions was guaranteed until 01 Aug 2015 as indicated by the sponsor, and the sponsor holds this responsibility.

The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.

Solvent used: 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 benzo(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 [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed. 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 (1st experiment):

Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains indicated above.

Pre-incubation assay (2nd experiment):

The test substance / 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; 33, 100, 333, 1000, 2500 and 5000 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: 28-Jan-2014 to 07-Feb-2014

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar 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 Merckoplate® plates (minimal glucose agar plates).

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S 9 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. Revertant colonies were counted. 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. at least 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 stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically ([see 2014/1095075 Becker M.,Kamp H. 2014 c]).

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 (decrease in the number of his^+ revertants) was occasionally observed in the standard plate test depending on the strain and test conditions from about 333 µg/plate onwards. In the pre-incubation assay bacteriotoxicity (reduced his- or trp- background growth, decrease in the number of his^+ or trp^+ revertants) was observed depending on the strain and test conditions from about 2 500 µg/plate onwards.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the pre-incubation experiments with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested (see Table 5.8.1-19). Test substance precipitation was found in the standard plate test at 5000 µg/plate without S9 mix and from about 1000 µg/plate onward with S9 mix.

Table 5.8.1-19: Bacterial gene mutation assay with Reg. No. 5916256 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
1st experiment: Plate incorporation assay										
Metabol. Activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	31.3	16.7	70.0	54.7	18.3	12.0	9.0	9.7	65.0	69.0
5916256										
33 µg/plate	27.3	16.3	70.7	51.7	18.7	11.7	10.0	7.7	72.3	63.7
100 µg/plate	29.0	13.3	69.0	58.7	13.0	11.0	9.0	10.0	67.7	69.0
333 µg/plate	26.0	13.7	66.3	60.7	8.7	10.7	7.3	7.0	75.3	64.0
1000 µg/plate	23.3P	11.7	75.7P	64.7	13.7P	5.3	7.3P	6.7	69.7P	62.3
2500 µg/plate	23.0P	12.0	74.0P	54.0	11.3P	8.5C	6.3P	5.7	74.3P	65.7
5000 µg/plate	16.0P	6.3P	74.7P	18.0P	12.0P	13.0P	4.3P	4.3P	51.3P	64.0P
Pos. control§	1575.0	336.0	2444.3	5296.7	315.7	6112.3	169.3	2095.3	193.0	873.7
2nd experiment: Pre-incubation assay										
Metabol. Activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	19.3	17.0	47.0	34.3	10.3	9.3	8.7	7.7	46.7	58.7
5916256										
33 µg/plate	20.7	18.0	55.0	46.7	10.7	8.3	7.7	9.0	55.3	61.0
100 µg/plate	24.7	17.3	55.7	46.3	8.3	9.3	11.3	5.0	60.3	60.7
333 µg/plate	20.0	18.0	51.7	39.7	9.7	9.3	9.7	7.0	64.0	65.3
1000 µg/plate	23.7	17.7	61.3	42.3	10.0	11.0	6.7	7.7	45.7	55.7
2500 µg/plate	20.3	11.3	30.0	23.3	5.7	7.7	5.0	4.0	29.7	45.0
5000 µg/plate	10.0B	11.0B	12.0B	28.3B	7.3B	6.7B	4.3B	3.7B	20.7B	46.0B
Pos. control§	473.0	404.7	349.7	922.3	197.3	1497.7	259.3	1172.0	232.3	951.3

§: Compound and concentrations see Material and Methods (I.A.2.) above
B: Reduced background growth
C: Contamination
P: Precipitation

III. CONCLUSION

According to the results of the present study, the test substance Reg. No. 5916256 is <u>not</u> mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions chosen here.

Report: CA 5.8.1/8
Anonymous, 2014
Reg.No. 5916256 (Metabolite of BAS 610 F, iprodione)
IN VITRO MICRONUCLEUS ASSAY IN V79 CELLS (CYTOKINESIS BLOCK METHOD)
2014/1098024
Guidelines: OECD 487 Commission Regulation (EC) No 640/2012; B.49
GLP: Yes

Executive Summary
Preliminary results indicate non-genotoxicity. The final report will be available after submission. (expected date: June 2014)

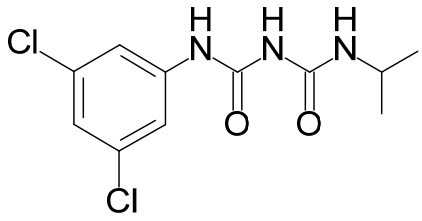
Assessment of genotoxicity for M610F007 (Reg.No. 5916256)

M610F007 does not cause point mutations in bacterial cells nor does it induce micronuclei *in vitro*. Overall there is no evidence that M610F007 is genotoxic *in vitro*.

Toxicological evaluation of metabolite M610F007 (preliminary)

QSAR analysis predicted a limited alert for genotoxicity *in vitro* with *in silico* metabolic activation which is considered of no relevance. There is no evidence for genotoxicity of M610F007 *in vitro*. No structural similarity to any other metabolite is apparrent; therefore M610F007 is placed into a single metabolite assessment group (2B). **In conclusion, M610F007 is considered to be of no genotoxicological concern. Therefore the human health based reference value of 1.5µg/kg bw/d according to the TTC concept for compounds of Cramer Class III can be applied.**

Metabolite RP36221 Reg.No. 5079618



RP36221; Reg.No.5079618

RP36221 is a dioxoimidazolidine-opened metabolite of parent iprodione and determined in plant and soil. Whereas dioxoimidazolidine-ring hydrolysis can be an initial step in the metabolism of iprodione in plant, leading to the formation of RP36221, the metabolic route in rat is different. In rat, ring-dealkylation is the preferred reaction prior to ring-hydrolysis, thereby bypassing the formation of RP36221. Strucuturally closely related are RP36115 (de-alkylated form) and RP36114 (de-alkylated and hydroxylated form), both major rat metabolites. De-alkylation as well as hydroxylation of RP36221, common reactions in rat metabolism of iprodione (e.g. turnover of parent iprodione to RP32490 for de-alkylation and formation of RP36114 by hydroxylation of RP36115) may lead to formation of RP36115 and RP36114. Therefore, all three metabolites are placed into a common assessment group (2C), as agreed with the RMS. As there might be potential human consumer exposure via the food chain this metabolite was considered for toxicological relevance assessment.

Structural Alert for RP36221

OASIS-Times gave a negative prediction of genotoxicity for parent metabolite RP37176 but an in domain positive prediction after *in silico* metabolism for a DNA-reactive hydroxyal-amine. This hypothetical occurring mutagenic compound potentially emerges after hydrolysis of the dichlorophenyl-ring closest amid-group, giving rise to 3,5-dichloroanline and subsequently to a hydroxylamine. However, total predictivity was out of domain mutagen. Similar predictions for hypothetical occuring hydroxylamines or C-nitroso compounds, of which hydroxyl-amines are precursors of, are proposed for 3,5-dichloroaniline, RP37176, M610F007 and RP44247. 3,5-dinitroaniline as well as RP37176, M610F007 and RP44247 were tested negative for genotoxicity. Therefore the identified alert is not relevant.
CAESAR predicted RP36221 as out of domain mutagen based on potential formation of metabolite CAS 330-54-1 (predicted DNA-reactive urea-derivative (NC=ON)). However, the underlying database for this prediction is poor, as the substance is clearly outside the applicability domain (global AD index 0.62; concordance 0.338). SarPy predicted RP36221 as non-mutagen potentially out of the applicability domain (global AD index 0.729; concordance 0.662).

In conclusion in two of the structure activity evaluation tools employed there was a limited alert for genotoxicity *in vitro* after *in silico* metabolic activation which is considered of no relevance based on similar predictions for 3,5-dichloroaniline, RP37176, M610F007 and RP44247 for which *in vitro* genotoxicity data indicate non-mutagenicity. Moreover, *in vitro* genotoxicity for RP36221 indicates non-mutagenicity (BASF DocID 2013/1286168).

Acute toxicity studies for metabolite RP36221

Report: CA 5.8.1/9
[redacted]., 2013a
Reg.No. 5079618 (metabolite of BAS 610 F, iprodione) - Acute oral toxicity study in rats
2013/1168382

Guidelines: OECD 423 (2001), (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, EPA 870.1100, JMAFF No 12 Nosan No 8147

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

Executive Summary

In an acute oral toxicity study, single doses of 2000 and 300 mg/kg body weight of Reg.No. 5079618 (Metabolite of BAS 610 F, iprodione; batch L80-172, purity: 98.4%) in corn oil were given to 3 administration groups of three fasted female animals each, (300 mg/kg in 3 females, 2000 mg/kg in 6 females) by gavage in a sequential manner. Animals were observed for 14 days. No mortality occurred at both dose levels. 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. One animal of the first 2000 mg/kg bw test group and one animal of the single 300 mg/kg bw test group showed stagnation of body weight during the second post-exposure week. Clinical observation in the 2000 mg/kg bw administration groups revealed impaired general state and piloerection in all animals and were observed from hour 0 to day 1 after administration. In the 300 mg/kg administration group, no clinical signs were observed. No animal showed any abnormal finding at necropsy. 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 2013/1168382)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 5079618 (Metabolite of BAS 610 F, iprodione); RP36221

Description:	Solid, white
Density:	not given
Lot/Batch #:	L80-172
Purity/content:	98.4% (tolerance +/- 1.0 %)
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: Corn oil Ph.Eur

3. Test animals:

Species:	Rat
Strain:	Wistar / Crl:WI (Han) SPF
Sex:	female
Age:	approximately 10 weeks
Weight at dosing:	173 - 185 g
Source:	Charles River Wiga GmbH, Sandhofer Weg 7, 97633 Sulzfeld, Germany
Acclimation period:	At least 5 days
Diet:	VRF1(P); SDS Special Diets Services, 67122 Altrip, Germany)
Water:	Tap water, ad libitum
Housing:	Single housing in Makrolon cages, type III
Environmental conditions:	
Temperature:	22 ± 3°C
Humidity:	30 - 70 %
Air changes:	approximately 10/hour
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: 24-Jun-2013 - 29-Jul-2013

2. Animal assignment and treatment:

Single doses of 2000 and 300 mg test substance/kg bw in corn oil were given to 3 administration groups of three fasted female animals each 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 at least once each workday. 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 5.8.1-20).

Table 5.8.1-20: Mortality of rats administered Reg. 5079618 by the oral route

Step No.	Sex	Dose (mg/kg bw)	Mortality / animals treated
1	Females	300	0/3
2	Females	2000	0/3
3	Females	2000	0/3

B. CLINICAL OBSERVATIONS

Clinical observation in the 2000 mg/kg bw administration groups revealed impaired general state and piloerection in all animals and were observed from hour 0 to day 1 after administration. In the 300 mg/kg administration group, no clinical signs were observed

C. BODY WEIGHT

The mean body weight increased within the normal range throughout the study period. One animal of the first 2000 mg/kg bw test group and one animal of the single 300 mg/kg bw test group showed stagnation of body weight during the second post-exposure week.

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. 5079618 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.

Assessment of acute toxicity for RP36221

RP36221 is of comparable acute oral toxicity in rats to parent iprodione.

Genotoxicity studies for metabolite RP36221

Report:	CA 5.8.1/10 Woitcowiak C., 2013a Reg.No. 5079618 (Metabolite of BAS 610 F, Iprodione) - Salmonella typhimurium/Escherichia coli reverse mutation assay 2013/1286168
Guidelines:	OECD 471 (1997), EPA 870.5100, (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
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium and E. coli were exposed to Reg. No. 5079618 (Batch L80-172; purity 98.4%) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in five independent sets of experiments. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment. In the standard plate test (plate incorporation assay), the test substance was tested at 33, 100, 333, 1000, 2500 and 5000 µg/plate in the absence and presence of metabolic activation in the first experiment in all tester strains and at 0.33, 1, 3.3, 10, 33, 100 and 333 with and without metabolic activation in S. typhimurium strains in the second experiment. In the pre-incubation assay, all strains were treated with 1, 3.3, 10, 33, 100 and 333 µg/plate in the absence and presence of metabolic activation (Experiment III and IV). Due to strong toxicity observed in the S. typhimurium strains, a further pre-incubation test was conducted in these strains at lower concentrations of 0.033 to 10 µg/plate without metabolic activation (Experiment V). A strong bacteriotoxic effect was observed in the standard plate test depending on the strain and test conditions from about 33 µg/plate onward and in the pre-incubation assay depending on the strain and test conditions from about 3.3 µg/plate onward. Neither in the plate incorporation nor in the pre-incubation experiments with and without metabolic activation a biologically relevant increase in number of revertants was observed in any strain tested. The negative and 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. 5079618 is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2013/1286168)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 5079618 (Metabolite of BAS 610 F, iprodione)

Description: Solid, white

Lot/Batch #: L80-172

Purity: 98.4% (tolerance +/- 1.0%)

Stability of test compound: The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 Feb 2015 as indicated by the sponsor, and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.

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

3. Activation:

S9 was produced from male Wistar rats. The rats were induced by intraperitoneal applications of 80 mg/kg bw phenobarbital and by oral administrations of 80 mg/kg bw β-naphthoflavone each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g. Aliquots of the supernatant were frozen and stored at -70 to -80 °C. The S9 mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction was thawed at room temperature and 1 volume of S9-fraction was 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:
(Triplicate plates were prepared for all test groups)

1st experiment:	0, 33, 100, 333, 1000, 2500 and 5000 µg/plate with and without S9 mix in TA 1535, TA 100, TA 1537, TA 98, E. coli WP2 uvrA
2nd experiment:	0, 0.33, 1.0, 3.3, 10, 33 and 100 µg/plate (without S9) 0, 1.0, 3.3, 10, 33, 100 and 333 µg/plate (with S9) in TA 1535, TA 100, TA 1537 and TA 98 (Reason: Bacteriotoxicity was observed in the 1st experiment)
Pre-incubation assay:	(Triplicate plates were prepared for all test groups)
3rd experiment:	0, 1.0, 3.3, 10, 33, 100 and 333 µg/plate (TA strains) 0, 33, 100, 333, 1000, 2500 and 5000 µg/plate (E.coli) with and without S9 mix in TA 1535, TA 100, TA 1537, TA 98, E. coli WP2 uvrA
4th experiment:	0 1.0, 3.3, 10, 33, 100 and 333 µg/plate without S9 mix in TA 98 (Reason: Due contaminations observed in the pre-incubation test with TA 98 an evaluation of this tester strain was not possible. The 3rd Experiment with TA 98 was not reported.)
5th experiment:	0, 0.033, 0.1, 0.33, 1.0, 3.3 and 10 µg/plate without S9 mix in TA 1535, TA 100, TA 1537 and TA 98 (Reason: Strong bacteriotoxicity was observed in the pre-incubation test in the Salmonella strains.)

B. TEST PERFORMANCE:

1. Dates of experimental work: 13-Aug-2013 - 05-Sep-2013

2. Plate incorporation assay:
To test tubes containing 2-mL portions of warm soft agar 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 were poured onto Merckoplate® plates (minimal glucose agar plates).

3. Pre-incubation assay:
100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S 9 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 were 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. Revertant colonies were counted. 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 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 were 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 stability of the test substance in the vehicle DMSO was verified analytically ([see 2014/1095068 Becker M.,Kamp H. 2013 b])

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay. A strong bacteriotoxic effect (reduced his^- background growth, decrease in the number of his^+ or trp^+ revertants, reduction in the titer) was observed in the standard plate test depending on the strain and test conditions from about 33 µg/plate onward. In the pre-incubation assay strong bacteriotoxicity (reduced his^- background growth, decrease in the number of his^+ revertants, reduction in the titer) was observed depending on the strain and test conditions from about 3.3 µg/plate onward.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the pre-incubation experiments with and without metabolic activation a biologically relevant increase in number of revertants was observed in any strain tested (seeTable 5.8.1-21; Table 5.8.1-22). The negative and positive controls yielded revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system. Test substance precipitation was found depending on the test conditions from about 3.3 µg/plate onward.

Table 5.8.1-21: Bacterial gene mutation assay with Reg. No. 5079618 - Mean number of revertants in plate incorporation tests

Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Experiment 1: Plate incorporation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	31	22	60	45	17	15	10	0B/P	67	57
Reg. No. 5079618										
33 µg/plate	26	10	67	20	14	10	8	0B/P	61	52
100 µg/plate	27	13	44	14	12	9	9	0B/P	66	55
333 µg/plate	16	9	53	14	10	8	9	0B/P	58	55
1000 µg/plate	15	5	39	11	11	7	4	0B/P	58	58
2500 µg/plate	10	4	39	11	9	7	3	0B/P	58	52
5000 µg/plate	6	5	29	8	7	7	2	0B/P	50	51
Pos. control[§]	1406	704	1523	1355	375	1226	524	649	452	699
Experiment 2: Plate incorporation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg.control (DMSO)	32	19	51	42	15	14	12	9	-	-
Reg. No. 5079618										
0.33 µg/plate	-	22	-	39	-	14	-	9	-	-
1 µg/plate	30	22	46	44	13	13	11	11	-	-
3.3 µg/plate	35	21	52	47	12	14	13	11	-	-
10 µg/plate	36	18	51	43	12	13	11	9	-	-
33 µg/plate	32	12	49	22	12	12	12	1	-	-
100 µg/plate	19	10	42	22	10	8	9	0B/P	-	-
333 µg/plate	20	-	40	-	8	-	8	-	-	-
Pos. control[§]	1347	749	1402	1242	-	1297	264	660	-	-

[§] = Compound and concentrations see Material and Methods (I.A.2.) above
B = reduced background growth
P = Precipitation

Table 5.8.1-22: Bacterial gene mutation assay with Reg. No. 5079618 - Mean number of revertants in pre-incubation tests

Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Experiment 3: Pre-incubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	-	-	49	53	12	11	7	8	37	81
Reg. No. 5079618										
1 µg/plate	-	-	53	50	11	10	8	9	39	83
3.3 µg/plate	-	-	44	40	12	8	6	7	35	78
10 µg/plate	-	-	40	26	11	5	9	3	39	80
33 µg/plate	-	-	45	0B	12	0B	5	0B	50	74
100 µg/plate	-	-	32	0B	11	0B	3	0B	42	76
333 µg/plate	-	-	32	0B	7	0B	0B	0B	47	69
Pos. control§	-	-	1452	1200	471	1457	253	535	252	1477
Experiment 4: Pre-incubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg.control (DMSO)	28	-	-	-	-	-	-	-	-	-
Reg. No. 5079618										
1 µg/plate	27	-	-	-	-	-	-	-	-	-
3.3 µg/plate	26	-	-	-	-	-	-	-	-	-
10 µg/plate	27	-	-	-	-	-	-	-	-	-
33 µg/plate	23	-	-	-	-	-	-	-	-	-
100 µg/plate	19	-	-	-	-	-	-	-	-	-
333 µg/plate	15	-	-	-	-	-	-	-	-	-
Pos. control§	763	-	-	-	-	-	-	-	-	-
Experiment 5: Pre-incubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg.control (DMSO)	-	19	-	41	-	11	-	8	-	-
Reg. No. 5079618										
0.033 µg/plate	-	19	-	44	-	10	-	8	-	-
0.1 µg/plate	-	17	-	35	-	11	-	9	-	-
0.33 µg/plate	-	18	-	37	-	11	-	9	-	-
1 µg/plate	-	18	-	40	-	11	-	9	-	-
3.3 µg/plate	-	14	-	29	-	9	-	9	-	-
10 µg/plate	-	5	-	20	-	7	-	2	-	-
Pos. control§	-	870	-	1173	-	766	-	609	-	-

§ = Compound and concentrations see Material and Methods (I.A.2.) above
B = reduced background growth
P = Precipitation

III. CONCLUSIONS

According to the results of the present study, the test substance Reg. No. 5079618 is <u>not</u> mutagenic in the Salmonella typhimurium/Escherichia coli reverse mutation assay under the experimental conditions chosen here.

Report:	CA 5.8.1/11 Wollny H.-E., 2013b Reg.No. 5079618 (metabolite of BAS 610 F, Iprodione): In vitro cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells 2013/1286166
Guidelines:	OECD 476 (1997), (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.17, EPA 870.5300, EPA 712-C-98-221
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

Reg. No. 5079618 (Metabolite of BAS 610 F; Batch: L80-172, Purity: 98.4%) was tested in vitro for its ability to induce forward mutations in L5178Y cells by assessing the mutation of the $TK^{+/-}$ locus. 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 48 µg/mL were used in the main experiments. The treatment intervals in the absence of metabolic activation were 4 and 24 h in the first and second experiment, respectively. The treatment interval for cultures treated with the test substance in the presence of metabolic activation was 4 h for both experiments. Methyl methanesulfonate (MMS) and cyclophosphamide (CPA) served as positive controls in the experiments without and with metabolic activation, respectively. Cytotoxic effects were observed in the first experiment at 18.0 µg/mL without metabolic activation following 4 hours of treatment. In the second experiment cytotoxic effects occurred at 18.0 µg/mL and above after 24 hours treatment without metabolic activation and at 48.0 µg/mL with metabolic activation. Precipitation at the end of treatment was noted in experiment II at 24.0 µg/mL without metabolic activation and at 48.0 µg/mL with metabolic activation. No substantial and reproducible dose dependent increase in mutant colony numbers was observed in both main experiments. No relevant shift of the ratio of small versus large colonies was observed up to the maximal concentration of the test item. The positive control substances, however, induced a marked increase in mutant frequency. Based on the results of the study it is concluded that under the conditions of the test Reg. No. 5079618 does not induce forward mutations in mammalian cells in-vitro.

(BASF DocID 2013/1286166)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Reg. No. 5079618 (Metabolite of BAS 610 F, iprodione)

Description: Solid, white

Lot/Batch #: L80-172

Purity: 98.4% (tolerance ± 1.0%)

Stability of test compound: Stable in DMSO

Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: A negative control was not employed in this study.

Solvent control: DMSO

Positive control -S9: Methyl methanesulfonate (MMS) 19.5 µg/mL

Positive control +S9: Cyclophosphamide (CPA) 3.0 and 4.5 µg/mL

3. Activation:

S9 was produced from the livers of induced 8-12 weeks old male Wistar [Hsd Cpb: WU] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthtoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3) parts followed by centrifugation at 9000 g.

An appropriate quantity of S9 supernatant was mixed with S9 cofactor solution to give a final protein concentration of 0.75 mg/mL in the cultures. Cofactors were added to the S9 mix to reach the following concentrations:

Component	Concentration
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
in 100 mM sodium-ortho-phosphate buffer (pH 7.4)	

4. Test organism:

The L5178Y cell line, which is characterized by a high proliferation rate (doubling time 10 - 12 h in stock cultures) and cloning efficiencies of untreated cells of usually more than 50%. The cells have a stable karyotype with a near diploid (40 ± 2) chromosome number. Large stocks of the cleansed L5178Y cell line were stored in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination and checked for karyotype stability and spontaneous mutant frequency. Prior to treatment, the amount of spontaneous mutants was reduced by growing the cells for one day in RPMI 1640-HAT medium supplemented with Hypoxanthine ($5.0x10^{-3}$M), Aminopterin ($2.0x10^{-5}$M), Thymidine ($1.6x10^{-3}$M) and Glycin ($5.0x10^{-3}$M) followed by a recovery period of 2 days in RPMI 1640 medium containing Hypoxanthine ($1.0x10^{-4}$M) and Thymidine ($1.6x10^{-3}$M). After this incubation the cells were returned to complete culture medium (see below).

5. Culture media:

Complete culture medium: RPMI 1640 medium supplemented with 15% horse serum (24 hour treatment, 3% HS during 4 hour treatment), 1% of 100 U/100 µg/mL Penicillin/Streptomycin, 220 µg/mL Sodium-Pyruvate, and 0.5 – 0.75% Amphotericin used as antifungal agent.

Selection medium: RPMI 1640 (complete culture medium) by addition of 5 µg/mL TFT

Saline G solution: Composition per litre (pH 7.2): NaCl 8000 mg, KCl 400 mg, Glucose 1100 mg, $Na_2HPO_4x2H_2O$ 192 mg, KH_2PO_4 150 mg

6. Locus examined: Thymidine Kinase Locus ($TK^{+/-}$)

7. Test concentrations:

a) Preliminary toxicity assay: Eight concentrations ranging from 23.4 to 3000 µg/mL

b) Mutation assay:

1st experiment: 0.38, 0.75, 1.5, 3.0, 6.0, 12.0, 18.0 and 24.0 µg/mL without metabolic activation

0.75, 1.5, 3.0, 6.0, 12.0, 18.0, 24.0 and 48.0 µg/mL with metabolic activation

2nd experiment: 0.75, 1.5, 3.0, 6.0, 12.0, 18.0 and 24.0 µg/mL without metabolic activation

3.0, 6.0, 12.0, 18.0, 24.0, 36.0 and 48.0 µg/mL with metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 25-Jun-2013 to 29-Jul-2013

2. Preliminary cytotoxicity assay:

A pre-test was performed in order to determine the concentration range of the mutagenicity experiments. PH value and osmolarity were determined at the maximal concentration of the test item and in the solvent control without metabolic activation. $1x10^7$ cells ($3x10^6$ cells at the beginning of 24 h treatment) were exposed to each concentration of the test item for 4 and 24 hours without and 4 hours with metabolic activation. Following treatment the cells were washed twice by centrifugation and resuspended in "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium for a 2-day growth period. The cell density was determined immediately after treatment and at each day of the growth period and adjusted to $3x10^5$ cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated at the end of the growth period.

3. Mutation Assay:

Cell treatment and expression: In the mutation experiment 1×10^7 ($3x10^6$ during 24 h exposure) cells/flask (80 cm^2 flasks) suspended in 10 mL RPMI medium were exposed to the test item concentrations either in the presence or absence of metabolic activation. Positive and solvent controls were performed in parallel. After 4 h (24 h in the second experiment) the test item was removed by centrifugation and the cells were washed twice with "saline G solution". Subsequently the cells were resuspended in 30 mL complete culture medium and incubated for an expression and growth period of totally 48 h. The cell density was determined each day and adjusted to 3×10^5 cells/mL, if necessary. The relative suspension growth (RSG) of the treated cell cultures was calculated by the day 1 fold-increase in cell number multiplied by the day 2 fold-increase in cell number.

Selection:	After the expression period the cultures were selected. Cells from each experimental group were seeded into 2 microtiter plates so that each well contained approximately 4×10^3 cells in selective medium with TFT. The viability (cloning efficiency) was determined by seeding about 2 cells per well into microtiter plates (same medium without TFT). The plates were incubated at 37 ± 1.5 °C in 4.5% CO_2/95.5% humidified air for 10 - 15 days. Then the plates were evaluated. The relative total growth (RTG) was calculated by the RSG multiplied by the viability.
Size distribution of the colonies:	Colonies were counted manually. In accordance with their size the colonies were classified into two groups. The colony size distribution was determined in the controls and at all concentrations of the test item. Criteria to determine colony size were the absolute size of the colony (more than 1/3 of a well for large colonies) and the optical density of the colonies (the optical density of the small colonies is generally higher than the optical density of the large ones).
Calculations:	**Pre-test** total suspension growth (4 h treatment): (cell number at 24 h / cell number at 4 h) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h) total suspension growth (24 h treatment): (cell number at 24 h / cell number of seeded cells per mL (100000)) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h) relative suspension growth: total suspension growth × 100 / total suspension growth of corresponding control

Main test

total suspension growth (4 h treatment):
(cell number at 24 h / cell number at 4 h) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h)

total suspension growth (24 h treatment):
(cell number at 24 h / cell number of seeded cells per mL (100000)) × (cell number at 48 h / if cell number at 24 h > 300000 then 300000, if cell number at 24 h < 300000 then cell number at 24 h) × (cell number at 72 h / if cell number at 48 h > 300000 then 300000, if cell number at 48 h < 300000 then cell number at 48 h)

relative suspension growth:
total suspension growth × 100 / total suspension growth of corresponding control

relative total growth:
relative suspension growth × relative cloning efficiency / 100

cloning efficiency (viability):
ln(mean number of empty wells per plate / 96) / cells seeded per well

relative cloning efficiency:
cloning efficiency × 100 / cloning efficiency of corresponding control

cells survived:
cloning efficiency × cell number seeded in TFT medium

mutant colonies / 106 cells:
small mutant colonies + large mutant colonies

threshold:
number of mutant colonies per 10^6 cells of each solvent control plus 126

cloning efficiency (viability):
cloning efficiency determined after the expression period to measure viability of the cells without selective agent

4. Statistics:

A linear regression (least squares) was performed to assess a possible dose-dependent increase of mutant frequencies using SYSTAT®11 (SYSTAT Software, Inc., 501, Canal Boulevard, Suite C, Richmond, CA 94804, USA) statistics software. The number of mutant colonies obtained for the groups treated with the test item was compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05. However, both, biological relevance and statistical significance were considered together.

5. Evaluation criteria:

The test item is considered mutagenic if at least one of the following criteria is met:

- The induced mutation frequency reproducibly exceeds a threshold of 126 colonies per 10^6 cells above the corresponding solvent control.
- A relevant increase of the mutation frequency should be dose-dependent and correspondingly statistically significant.
- A mutagenic response is considered to be reproducible if it occurs in both parallel cultures.

The test item is considered non-mutagenic if at least one of the following criteria is met:

- The mutation frequency is below a threshold of 126 colonies per 10^6 cells above the concurrent negative control value.
- No evidence of reproducibility of an increase in mutant frequencies is obtained.
- No statistical significant dose-related increase in mutant frequencies using an appropriate statistical trend.
- If the threshold is reproducibly exceeded but the increase of the mutation frequency is not dose dependent and a biological relevance of the effect can be excluded.

In the evaluation of the test results the historical variability of the mutation rates in the solvent controls of this study were taken into consideration. Results of test groups were generally rejected if the relative total growth was less than 10% of the vehicle control. Whenever a test item is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency is accompanied by a reproducible and dose dependent shift in the ratio of small versus large colonies clastogenic effects were indicated. A test item not meeting the conditions for a classification as mutagenic or non-mutagenic is considered equivocal in this assay and may be considered for further investigation.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Purity of the test item was verified by HPLC analysis (see BASF study report ASAP13_030; Annex III of the final study report). The stability of the test substance in the vehicle DMSO was verified analytically ([see 2014/1095068 Becker M.,Kamp H. 2013 b]).

B. PRELIMINARY CYTOTOXICITY ASSAY

The pre-experiment was performed in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation. Test item concentrations between 23.4 µg/mL and 3000 µg/mL (equal to a molar concentration of approximately 10 mM) were used. Cytotoxic effects leading to RSG values below 50% were observed down to the lowest concentration of 23.4 µg/mL in the presence and absence of metabolic activation following 4 and 24 hours treatment. Precipitation at the end of the treatment period was observed by the unaided eye at 23.4 µg/mL and above after 4 and 24 hours treatment without metabolic activation and at 46.9 µg/mL and above with metabolic activation (4 hours treatment). There was no relevant shift of the osmolarity and pH value even at the maximum concentration of the test item. The dose range of the main experiments was set according to data generated in the pre-experiment: the highest concentration tested in the mutagenicity experiments was 24.0 and 48.0 µg/mL without and with metabolic activation.

C. MUTAGENICITY ASSAYS

Precipitation at the end of treatment was noted in experiment II at 24.0 µg/mL without metabolic activation and at 48.0 µg/mL with metabolic activation (see Table 5.8.1-23 and Table 5.8.1-24). Relevant cytotoxic effects indicated by a relative total growth of less than 50% of survival in both parallel cultures were observed in the first experiment at 18.0 µg/mL without metabolic activation following 4 hours of treatment. In the second experiment cytotoxic effects as described above occurred at 18.0 µg/mL and above after 24 hours treatment without metabolic activation and at 48.0 µg/mL with metabolic activation. No substantial and reproducible dose-dependent increase of the mutation frequency was observed with and without metabolic activation. The mutation frequency did not reach or exceed the threshold of 126 above the corresponding solvent control. A significant dose-dependent trend of the mutation frequency was solely detected in the first culture of the first experiment without metabolic activation ($p<0.05$). Since the mutation frequency neither exceeded the historical range of solvent controls nor the threshold as indicated above, the statistical result is considered as biologically irrelevant fluctuation and was therefore not documented in the report table. The lowest solvent control values (33, 38, and 41 colonies per 10^6 cells) fell just short of the recommended 50 – 170 x 10^6 control range. However, the number of mutant colonies per 10^6 cells in the parallel cultures (71, 59, and 63 colonies per 10^6 cells) were fully acceptable. The positive controls MMS (19.5 µg/mL) and CPA (3.0 and 4.5 µg/mL) showed a distinct increase in induced total mutant colonies and an increase of the relative quantity of small versus large induced colonies thereby validating the sensitivity of the test. The relative total growth of the MMS control of the first culture of the second experiment without metabolic activation fell short of the lower acceptable limit of 10% (5.6%). The data are acceptable, as the relative suspension growth remained above this limit with 13.9%.

Table 5.8.1-23: Gene mutation in mammalian cells - 1st experiment

	Con. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment I / 4 h treatment			**Culture I**			**Culture II**		
Solv. Control with DMSO		-	100.0	33	159	100.0	71	197
Pos. Control with MMS	19.5	-	13.5	697	159	25.0	746	197
Test item	0.38	-	culture was not continued#			culture was not continued#		
Test item	0.75	-	culture was not continued#			culture was not continued#		
Test item	1.5	-	87.5	48	159	177.1	46	197
Test item	3.0	-	78.7	63	159	127.5	72	197
Test item	6.0	-	65.4	64	159	135.6	74	197
Test item	12.0	-	46.0	69	159	92.1	52	197
Test item	18.0	-	11.2	75	159	19.5	56	197
Test item	24.0 (p)	-	culture was not continued##			culture was not continued##		
Solv. Control with DMSO		+	100.0	38	164	100.0	59	185
Pos. Control with CPA	3.0	+	47.9	234	164	44.4	260	185
Pos. Control with CPA	4.5	+	34.5	312	164	55.3	277	185
Test item	0.75	+	culture was not continued#			culture was not continued#		
Test item	1.5	+	culture was not continued#			culture was not continued#		
Test item	3.0	+	91.6	57	164	95.0	71	185
Test item	6.0	+	95.3	67	164	72.2	85	185
Test item	12.0	+	93.6	31	164	82.1	40	185
Test item	18.0	+	85.9	43	164	86.5	43	185
Test item	24.0	+	58.1	46	164	59.6	35	185
Test item	48.0 (p)	+	culture was not continued##			culture was not continued##		

P = precipitation
threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
# culture was not continued as a minimum of only four concentrations is required by the guidelines
culture was not continued due to exceedingly severe cytotoxic effects

Table 5.8.1-24: Gene mutation in mammalian cells - 2nd experiment

	Con. µg/mL	S9 mix	relative total growth	mutant colonies/10^6 cells	threshold	relative total growth	mutant colonies/10^6 cells	threshold
Experiment II / 24 h treatment			**Culture I**			**Culture II**		
Solv. Control with DMSO		-	100.0	128	254	100.0	72	198
Pos. Control with MMS	19.5	-	5.6	380	254	10.1	299	198
Test item	0.75	-	culture was not continued#			culture was not continued#		
Test item	1.5	-	culture was not continued#			culture was not continued#		
Test item	3.0	-	76.1	106	254	69.5	81	198
Test item	6.0	-	68.4	111	254	89.5	65	198
Test item	12.0	-	64.4	71	254	50.8	101	198
Test item	18.0	-	30.6	94	254	34.7	72	198
Test item	24.0 (p)	-	19.9	107	254	17.9	70	198
Experiment II / 4 h treatment			**Culture I**			**Culture II**		
Solv. Control with DMSO		+	100.0	41	167	100.0	63	189
Pos. Control with CPA	3.0	+	65.2	157	167	54.2	361	189
Pos. Control with CPA	4.5	+	20.2	504	167	32.1	441	189
Test item	3.0	+	culture was not continued#			culture was not continued#		
Test item	6.0	+	culture was not continued#			culture was not continued#		
Test item	12.0	+	110.4	53	167	121.6	64	189
Test item	18.0	+	105.8	59	167	81.4	94	189
Test item	24.0	+	76.5	43	167	78.6	78	189
Test item	36.0	+	50.5	46	167	51.0	59	189
Test item	48.0 (p)	+	20.2	83	167	22.4	72	189

P = precipitation
threshold = number of mutant colonies per 10^6 cells of each solvent control plus 126
# culture was not continued as a minimum of only four concentrations is required by the guidelines

III. CONCLUSION

Based on the results of the study it is concluded that under the conditions of the test Reg. No. 5079618 does not induce forward mutations in the $TK^{+/-}$ locus in L5178Y cells in vitro.

Report:	CA 5.8.1/12 Bohnenberger S., 2013b Reg.No. 5079618 (metabolite of BAS 610 F, Iprodione) - In vitro micronucleus test in chinese hamster V79 cells 2013/1164782
Guidelines:	OECD 487 (2010), Commission Regulation EU No. 640/2012 of 06 July 2012 - B.49: In vitro Mammalian Cell Micronucleus Test
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

Reg. No. 5079618 (Batch L80-172; purity 98.4%) was tested *in vitro* for its potential to induce micronuclei in V79 cells of the Chinese hamster in the absence and presence of metabolic activation by S9 mix in two independent experiments. Concentrations of 5.8 to 2950 µg/mL were tested within an exposure period of 4 hours in a pre-test with and without metabolic activation. Precipitation was observed at all test substance concentrations, therefore a second pre-test was conducted with 0.1 to 300 µg/mL. Since no cytotoxicity was observed in both pres-tests, the second pre-test was designated Experiment I. In Experiment II the exposure period was 24 hours without metabolic activation and 4 hours with metabolic activation. Visible precipitation of the test item in the culture medium was observed microscopically at 2.6 µg/mL and above in the absence of S9 mix and at 40.0 µg/mL and above in the presence of S9 mix at the end of treatment in Experiment I. In addition, precipitation occurred in Experiment II at 15.1 µg/mL and above in the absence of S9 mix and at 16.0 µg/mL and above in the presence of S9 mix at the end of treatment. Cytotoxicity was observed only in the absence of metabolic activation at the highest evaluated concentration of 15.1 µg/mL in Experiment II. In both experiments, in the absence and presence of S9 mix, no biologically relevant increase in the number of cells carrying micronuclei was observed. Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test. Based on the results of this study, Reg. No. 5079618 is considered to not induce micronuclei in vitro in V79 cells in the presence or absence of metabolic activation.

(BASF DocID 2013/1164782)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg. No. 5079618 (Metabolite of BAS 610 F, iprodione)

Description: Solid, white

Lot/Batch #: L80-172

Purity: 98.4 % (tolerance ± 1.0 %)

Stability of test compound: Stable in DMSO (solvent)

Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: A negative control was not employed in this study

Solvent control: DMSO

Positive controls, -S9: Mitomycin C
(MMC, 0.3 µg/mL, dissolved in deionised water)
Griseofulvin (8.0 µg/mL, dissolved in deionised water)

Positive control, +S9: Cyclophosphamide
(CPA, 10.0 µg/mL, dissolved in saline)

3. Activation: S9 was produced from male Wistar rats. The rats were induced by intraperitoneal applications of 80 mg/kg bw phenobarbital and by oral administrations of 80 mg/kg bw β-naphthoflavone each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g. Aliquots of the supernatant were frozen and stored in ampoules at –80 °C. The S9 mix preparation was performed according to Ames test et al. Briefly, an appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution (see below) to result in a final protein concentration of 0.75 mg/mL in the cultures. During the experiment the S9 mix was stored on ice.

The S9-mix was prepared immediately before use and had the following composition:

Component	*Concentration*
Sodium-ortho-phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

4. Test organisms: Chinese hamster V79 cells

This is a continuous cell line with a population doubling time of 13 hours.

5. Culture medium/conditions: About 5 x 10^5 cells/flask were seeded in 15 mL of MEM (minimal essential medium) containing Hank's salts, glutamine, Hepes (25 mM), penicillin/ streptomycin (100 U/mL/100 mg/mL) and 10 % (v/v) fetal bovine serum (FBS). Cell cultures were incubated at 37 °C in a humidified atmosphere with 1.5% carbon dioxide (98.5% air).

Exponentially growing stock cultures more than 50 % confluent were rinsed with Ca-Mg-free salt solution. Afterwards the cells were treated with trypsin-EDTA-solution at 37 °C for approx. 5 minutes. By adding complete culture medium including 10 % (v/v) FBS the enzymatic treatment was stopped and a single cell suspension was prepared. The cells were seeded into Quadriperm dishes, which contained microscopic slides. Into each chamber 1.0 x 10^5 – 1.5 x 10^5 cells were seeded with regard to the preparation time. In each experimental group two parallel cultures were set up.

6. Test concentrations:

a) Preliminary toxicity assay: Experiment I*:

5.8 - 2950.0 µg/mL with and without metabolic activation

*Since precipitation was observed at all test concentrations, a second pre-test was conducted.

Experiment I:

0.1 - 300.0 µg/mL with and without metabolic activation

This preliminary test was designated Experiment I (see Table 5.8.1-25).

b) Cytogenicity assay: Experiment II:

0.4 - 2950.0 µg/mL without metabolic activation

0.1 - 300.0 µg/mL with metabolic activation

Table 5.8.1-25: Doses applied in the Micronucleus Test in Chinese Hamster V79 Cells with Reg.No. 5079618 (Metabolite of BAS 610 F, iprodione)

Preparation interval	Exposure period	Exp.	Concentration in µg/mL									
			Without S9 mix									
24 hrs	4 hrs	I*	5.8[P]	11.5[P]	23.0[P]	46.1[P]	92.2[P]	184.4[P]	368.8[P]	737.5[P]	1475.0[P]	2950.0[P]
24 hrs	4 hrs	I	0.1	0.2	**0.4**	**1.0**	**2.6[P]**	6.4[P]	16.0[P]	40.0[P]	100.0[P]	300.0[P]
24 hrs	24 hrs	II	0.4	1.0	**2.4**	**6.0**	**15.1[P]**	37.8[P]	75.7[P]	188.8[P]	472.0[P]	1180.0[P]
												2950.0[P]
			With S9 mix									
24 hrs	4 hrs	I*	5.8[P]	11.5[P]	23.0[P]	46.1[P]	92.2[P]	184.4[P]	368.8[P]	737.5[P]	1475.0[P]	2950.0[P]
24 hrs	4 hrs	I	0.1	0.2	0.4	1.0	2.6	**6.4**	**16.0**	**40.0[P]**	100.0[P]	300.0[P]
24 hrs	4 hrs	II	0.1	0.2	0.4	1.0	**2.6**	**6.4**	**16.0[P]**	40.0[P]	100.0[P]	300.0[P]

Evaluated experimental points are shown in bold characters
[P] Precipitation was observed microscopically at the end of treatment
* Was repeated due to precipitation at all applied concentrations

B. TEST PERFORMANCE:

1. Dates of experimental work: 10-Jun-2013 - 17-Jul-2013

2. Preliminary cytotoxicity assay: With respect to the molecular weight and the purity of the test item, 2950.0 µg/mL of Reg. No. 5079618 was applied as top concentration for treatment of the cultures in a pre-test. Test item concentrations between 5.8 and 2950.0 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity.

3. Cytogenicity Assay:

Exposure period 4 hours: The culture medium of exponentially growing cell cultures was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL medium was added. Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed twice 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. Then the cells were cultured in complete medium containing 10% (v/v) FBS for the remaining culture time of 20 hours.

Exposure period 24 hours: The culture medium of exponentially growing cell cultures was replaced with complete medium containing 10% (v/v) FBS including the test item without S9 mix. The medium was not changed until preparation of the cells. Concurrent solvent and positive controls were performed.

Preparations of cultures: For the micronucleus analysis, 24 hours after the start of the exposure, the cells were treated on the slides in the chambers of the quadriperm dishes with deionised water for 1 to 1.5 min at 37 °C. Afterwards the cells were fixed twice with a solution containing 3 parts ethanol, 1 part acetic acid and 1.25 % (v/v) formaldehyde. After preparation the cells were stained with Giemsa and labelled with a computer-generated random code to prevent scorer bias.

Analysis of micronuclei and cytotoxicity: Evaluation was performed manually using microscopes with 40x objectives. The micronuclei were counted in cells showing a clearly visible cytoplasm area. The area of the micronucleus should not extend the third part of the area of the main nucleus. 1000 cells in two parallel cultures were scored for micronuclei, so that at least 2000 cells from clones with 2 - 8 cells were analysed per test group. The frequency of micronucleated cells was reported as % micronucleated cells. Cytotoxicity was assessed via counting the number of clones consisting of 1 cell (c1), 2 cells (c2), 3 - 4 cells (c4), and 5 - 8 cells (c8) among the cells that were scored for the presence of micronuclei. These clusters represented the cells that have divided 1, 2, or 3 times within the experiment. From these data, a proliferation index (PI) was calculated (see formula below). Only those cultures were evaluated which showed a PI > 1.3, in order to guarantee for a sufficient cell proliferation during treatment and recovery.

$$PI = \frac{(c1 \times 1) + (c2 \times 2) + (c4 \times 3) + (c8 \times 4)}{(c1 + c2 + c4 + c8)}$$

PI: Proliferation index
cx: Number of clones with x cells (with x: 1, 2, 4, or 8)

4. Statistics:

Statistical significance at the five per cent level was evaluated by means of the Chi-square test. Evaluation was performed only for test groups showing a higher number of micronucleated cells than the respective solvent control group.

5. Evaluation criteria:

A test item was classified as mutagenic if:

- the number of micronucleated cells is not in the range of the historical 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.

A test item can be classified as non-mutagenic if:

- the number of micronucleated cells in all evaluated test groups is in the range of the historical control data and
- no statistically significant or concentration-related increase in the number of micronucleated cells is observed in comparison to the respective solvent control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

Purity of the test item was verified by HPLC analysis (see BASF study report ASAP13_030; Appendic III of the final study report). The stability of the test substance in the vehicle DMSO was verified analytically ([see 2014/1095068 Becker M.,Kamp H. 2013 b]).

B. PRELIMINARY CYTOTOXICITY ASSAY:

In the pre-tests no cytotoxicity was observed at the evaluated concentrations in the absence and presence of S9 mix.

C. CYTOGENICITY ASSAYS:

Two independent experiments were performed. In Experiment I, the exposure period was 4 hours with and without S9 mix. In Experiment II, the exposure periods were 4 hours with S9 mix and 24 hours without S9 mix. The cells were prepared 24 hours after start of treatment with the test item. No cytotoxicity was observed in Experiment I. In Experiment II, only in the absence of S9 mix, clear cytotoxicity was observed at the highest evaluated concentration of 15.1 µg/mL. In Experiment I, visible precipitation of the test item in the culture medium was observed microscopically at 2.6 µg/mL and above in the absence of S9 mix and at 40.0 µg/mL and above in the presence of S9 mix at the end of treatment. In addition, precipitation occurred in Experiment II at 15.1 µg/mL and above in the absence of S9 mix and at 16.0 µg/mL and above in the presence of S9 mix at the end of treatment. In both experiments, in the absence and presence of S9 mix, no biologically relevant increase in the number of cells carrying micronuclei was observed (Table 5.8.1-26). The micronucleus rates of the cells after treatment with the test item (0.35 – 0.90 % micronucleated cells) were close to the range of the solvent control values (0.40 – 0.85 % micronucleated cells) and within the range of the laboratory historical control data. Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test.

Table 5.8.1-26: Summary of results of micronucleus test with Reg. No. 5079618 (Metabolite of BAS 610 F, iprodione)

Exp.	Preparation interval	Test item concentration [µg/mL]	Proliferation index	Micronucleated Cells* [%]
Exposure period 4 hrs without S9 mix				
I	24 hrs	Solvent control[1]	2.89	0.85
		Positive control[2]	2.00	**10.35[S]**
		0.4	2.96	0.50
		1.0	2.93	0.60
		2.6[P]	2.90	0.60
		300.0[P]	2.54	n.d.
Exposure period 24 hrs without S9 mix				
II	24 hrs	Solvent control[1]	3.18	0.40
		Positive control[3]	2.70	**9.05[S]**
		2.4	2.97	0.80
		6.0	2.59	0.50
		15.1[P]	1.80	0.40
Exposure period 4 hrs with S9 mix				
I	24 hrs	Solvent control[1]	1.94	0.80
		Positive control[4]	1.47	**6.55[S]**
		6.4	2.09	0.35
		16.0	2.22	0.55
		40.0[P]	1.85	0.85
		300.0[P]	1.89	n.d.
II	24 hrs	Solvent control[1]	2.17	0.85
		Positive control[4]	1.62	**6.05[S]**
		2.6	2.35	0.90
		6.4	2.31	0.75
		16.0[P]	2.23	0.65

* The total number of micronucleated cells was determined in a sample of 2000 cells
n.d. Not determined
[S] Number of micronucleated cells statistically significantly higher than corresponding control values
[P] Precipitation occurred microscopically at the end of treatment
[1] DMSO 0.5 % (v/v)
[2] Mitomycin C 0.3 µg/mL
[3] Griseofulvin 8.0 µg/mL
[4] CPA 10.0 µg/mL

III. CONCLUSIONS

Based on the results of the study, Reg. No. 5079618 is considered as non-mutagenic in this in vitro micronucleus test, when tested up to cytotoxic and/or precipitating concentrations.

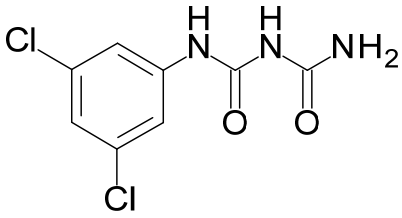
Assessment of genotoxicity for RP36221

RP36221 does not cause point mutations in bacterial cells nor does it induce micronuclei nor point mutations in eukaryotic cells *in vitro*. Overall there is no evidence that RP36221 is genotoxic *in vitro*.

Toxicological evaluation of metabolite RP36221

QSAR analysis predicted a limited alert for genotoxicity *in vitro* with *in silico* metabolic activation which is considered of no relevance. Acute toxicity after oral application to rats is comparable to parent iprodione. There is no evidence for genotoxicity *in vitro*. RP36221, RP36115 and RP36114 are placed into a common assessment group (2C), based on close structural similarity. Most likely all of three metabolites participate in the same metabolic pathway in rats after exposure to RP36221. **In conclusion, the toxicological properties of RP36221, assessed with major rat metabolites RP36115 and RP36114 in a common metabolite group 2C, are considered covered by parent iprodione based on the combined group exposure of the metabolites in rat metabolism. Therefore the human health based reference values for iprodione also apply to RP36221.**

Metabolite RP36115 Reg.No. 5079624



RP36115; Reg.No.5079624

RP36115 is a dioxoimidazolidine-opened metabolite of parent iprodione and determined in plant, livestock and rat. RP36115 is considered a major rat metabolite (see chapter MCA 5.1). Close structural similarity is apparent to RP36114, a hydroxylated from participating in the same metabolic pathway and also a major metabolite in rat. Close structural similarity is also given for RP36221, a potential pre-cursor metabolite (see section on RP36221) which is not a rat metabolite after exposure to iprodione. All three metabolites are placed into a common assessment group (Group 2C), as agreed with the RMS. As there might be potential human consumer exposure via the food chain metabolite RP36115 was considered for toxicological relevance assessment.

Structural Alert for RP36115

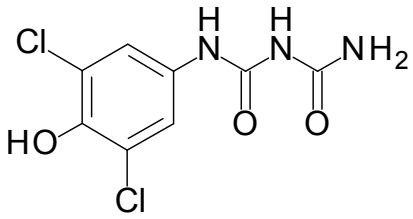
OASIS-Times gave a negative prediction of parent metabolite RP36115 genotoxicity but an in domain positive prediction after *in silico* metabolism for a DNA-reactive C-nitroso compound. This hypothetical occurring mutagenic compound potentially emerges after hydrolysis of the dichlorophenyl-ring closest amide-group, giving rise to 3,5-dichloroanline and subsequently to the predicted mutagen. However, total predictivity was out of domain mutagen. Similar predictions for hypothetical occurring metabolites with C-nitroso compounds are proposed for 3,5-dichloroaniline, M610F007 and RP44247. 3,5-dinitroaniline as well as M610F007 and RP44247 were tested negative for genotoxicity. Therefore the identified alert is not relevant.
CAESAR predicted RP36115 as out of domain mutagen based on potential formation of metabolite CAS 330-54-1 (predicted DNA-reactive urea-derivative (NC=ON)). However, the underlying database for this prediction is poor, as the substance is clearly outside the applicability domain (global AD index 0.688; concordance 0.343). SarPy predicted RP36115 as non-mutagen potentially out of the applicability domain (global AD index 0.729; concordance 0.657).

In conclusion in two of the structure activity evaluation tools employed there was a limited alert for genotoxicity *in vitro* after *in silico* metabolic activation which is considered of low relevance based on similar prediction for 3,5-dichloroaniline, M610F007 and RP44247 for which *in vitro* genotoxicity data indicate non-mutagenicity. Moreover, *in vitro* genotoxicity studies for structurally closely related RP36221 indicate non-mutagenicity. Overall there is no evidence that RP36115 is of genotoxic concern.

Toxicological evaluation of metabolite RP36115

QSAR analysis predicted a limited alert for genotoxicity *in vitro* with *in silico* metabolic activation which is considered of no relevance. RP36115 is considered a major rat metabolite (see chapter MCA 5.1). Previously, a non-guideline mechanistic study *in vitro* conducted side-by-side with parent iprodione was performed and reported in the original dossier from 1997 (BASF DocID C029154), indicating that RP36115 may inhibit testosterone secretion in porcine Leydig cells at comparable concentrations to parent iprodione. Another mechanistic study, not previously reported, indicated that RP36115 does not interact with the androgen receptor (BASF DocID R014678). Based on the close structural similarity between RP36115, RP36114 and RP36221, all metabolites are grouped into a common assessment group 2C. **In conclusion, the toxicological properties of RP36115, assessed with RP36114 and RP36221 in a common metabolite group 2C, are considered covered by parent iprodione based on the combined group exposure of the metabolites in rat metabolism. Therefore the human health based reference values for iprodione also apply to RP36115.**

Metabolite RP36114 Reg.No. 5079627



RP36114; Reg.No.5079627

RP36114 is a dioxoimidazolidine-opened metabolite of parent iprodione and determined in plant, livestock and rat. RP36114 is considered a major rat metabolite (see chapter 5.1). Close structural similarity is apparent to RP36115, a non-hydroxylated from participating in the same metabolic pathway in rat and also a major rat metabolite. Close structural similarity is also given for RP36221, a potential pre-cursor metabolite (see section on RP36221) which is not a rat metabolite after exposure to iprodione. All three metabolites are placed into a common assessment group (2C), as agreed with the RMS. As there might be potential human consumer exposure via the food chain metabolite RP36114 was considered for toxicological relevance assessment.

Structural Alert for RP36114

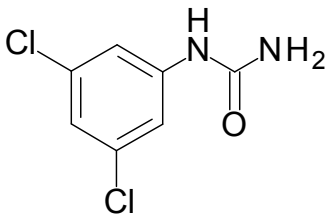
OASIS-Times gave a negative prediction of genotoxicity for parent metabolite RP36114 but an in domain positive prediction after *in silico* metabolism for a Quinoneimine. This hypothetical occurring mutagenic compound potentially emerges after hydrolysis of the dichlorophenyl-ring closest amide-group, giving rise to the predicted mutagen. However, total predictivity was out of domain mutagen. Similar mutagen predictions are evident for 3,5-dichloroaniline, where a phenyleneamine is predicted, the oxidized form of the Quinoneimine. 3,5-dinitroaniline was tested negative for genotoxicity. Therefore the identified alert is not relevant.
CAESAR predicted RP36114 as out of domain mutagen based on potential formation of metabolite CAS 330-54-1 (predicted DNA-reactive urea-derivative (NC=ON)). However, the underlying database for this prediction is poor, as the substance is clearly outside the applicability domain (global AD index 0.67; concordance 0.341). SarPy predicted RP36114 as non-mutagen potentially out of the applicability domain (global AD index 0.712; concordance 0.659).

In conclusion in two of the structure activity evaluation tools employed there was a limited alert for genotoxicity *in vitro* after *in silico* metabolic activation which is considered of no relevance due to RP36114 being a major rat metabolite and genotoxicity considered covered by testing of parent iprodione. Moreover similar mutagen prediction is evident for 3,5-dichloroaniline which was tested for genotoxicity, indicating non-mutagenicity. *In vitro* genotoxicity studies for structurally closely related RP36221 also indicate non-mutagenicity. Overall there is no evidence that RP36114 is of genotoxic concern.

Toxicological evaluation of metabolite RP36114

QSAR analysis predicted a limited alert for genotoxicity *in vitro* with *in silico* metabolic activation which is considered of no relevance. RP36115 is considered a major rat metabolite (see chapter 5.1). In a mechanistic study, not previously reported, it is indicated that RP36114 does not interact with the androgen receptor (BASF DocID R014678). Based on close structural similarity between RP36114, RP36115 and RP36221, all metabolites are grouped into a common assessment group 2C. **In conclusion, the toxicological properties of RP36114, assessed with RP36115 and RP36221 in a common metabolite group 2C, are considered covered by parent iprodione based on the combined group exposure of the metabolites in rat metabolism. Therefore the human health based reference values for iprodione also apply to RP36114.**

Metabolite RP44247 Reg.No. 89517



RP44247; Reg.No.89517

RP44247 is a dioxoimidazolidine-opened metabolite of parent iprodione and determined in plant, livestock and soil (photolysis) but not in rats, likely due to the ready elimination of precursor metabolite RP36115 in rat urine. Allthough RP44247 could potentially be formed in rat from RP36115 by de-amidation, a common metabolic process in rat, RP44247 was placed into a separate metabolite group (2E) as proposed by RMS. As there might be potential human consumer exposure via the food chain this metabolite was considered for toxicological relevance assessment.

Structural Alert for RP44247

OASIS-Times gave a negative prediction of parent metabolite RP44247 genotoxicity but an in domain positive prediction after *in silico* metabolism for a DNA-reactive C-nitroso compound. This hypothetical occurring mutagenic compound potentially emerges after hydrolysis of the dichlorophenyl-ring closes amide-group, giving rise to 3,5-dichloroanline and subsequently to the predicted mutagen. However, total predictivity was out of domain mutagen. Similar predictions for hypothetical occurring metabolites with C-nitroso compounds are proposed for 3,5-dichloroaniline and M610F007 which are both tested Ames negative, also after metabolic activation.
CAESAR predicted RP44247 as out of domain mutagen based on potential formation of metabolite CAS 330-54-1 (predicted DNA-reactive urea-derivative (NC=ON)). However, the underlying database for this prediction is poor, as the substance is clearly outside the applicability domain (global AD index 0.67; concordance 0.341). SarPy predicted RP44247 as non-mutagen potentially out of the applicability domain (global AD index 0.712; concordance 0.659).

In conclusion in two of the structure activity evaluation tools employed there was a limited alert for genotoxicity *in vitro* after *in silico* metabolic activation which is considered of no relevance based on similar predictions for 3,5-dichloroaniline and M610F007 for which *in vitro* genotoxicity data indicate non-mutagenicity. Moreover, *in vitro* genotoxicity data for RP44247 indicates non-mutagenicity (BASF DocID 2013/1282481).

Genotoxicity studies for metabolite RP44247

Report:	CA 5.8.1/13 Woitkowiak C., 2013a Reg.No. 89517 (metabolite of BAS 610 F, Iprodione) - Salmonella typhimurium / Escherichia coli reverse mutation assay 2013/1282481
Guidelines:	(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 No. L 142, (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.14 No. L 142, EPA 870.5100, OECD 471 (1997)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium and E. coli were exposed to Reg. No. 89517 (Batch OB0002; purity 99.9%) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in three independent sets of 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, the test substance was tested at 33, 100, 333, 1000, 2500 and 5000 µg/plate in the absence and presence of metabolic activation. In the second experiment (plate incorporation assay), S. typhimurium strains were treated with 1 to 333 µg/plate in the absence of metabolic activation. In the pre-incubation test, concentrations of 1, 3.3, 33, 100 and 333 µg/plate were used with and without metabolic activation. A strong bacteriotoxic effect was observed in the standard plate test depending on the strain and test conditions from about 100 µg/plate onward and in the pre-incubation assay from about 33 µg/plate onward. No test substance precipitation was found with and without S9 mix. Neither in the first and second experiment (plate incorporation tests) nor in the third experiment (pre-incubation test) a biologically relevant increase in the number of revertant colonies was noticed in any of the strains tested in presence or absence of metabolic activation. The negative and 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. 89517 is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2013/1282481)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 89517 (Metabolite of BAS 610 F, iprodione)

Description: Solid, white

Lot/Batch #: OB0002

Purity: 99.9% (tolerance +/- 1.0%)

Stability of test compound: The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 Nov 2019 as indicated by the sponsor, and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.

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

3. Activation:

S9 was produced from male Wistar rats. The rats were induced by intraperitoneal applications of 80 mg/kg bw phenobarbital and by oral administrations of 80 mg/kg bw β-naphthoflavone each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g. Aliquots of the supernatant were frozen and stored at -70 to -80 °C. The S9 mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction was thawed at room temperature and 1 volume of S9-fraction was 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:	Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) (Experiment I); neg. control; 1, 3.3, 10, 33, 100 and 333 µg/plate and positive control without S9 (Experiment II)
Pre-incubation assay:	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; 1, 3.3, 10, 33, 100 and 333 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9).

B. TEST PERFORMANCE:

1. Dates of experimental work: 02-Jul-2013 - 11-Jul-2013

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar 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 were poured onto Merckoplate® plates (minimal glucose agar plates.

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S 9 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 were 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. Revertant colonies were counted. 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 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 is 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 stability of the test substance in the vehicle DMSO was verified analytically ([see 2014/1095065 Becker M.,Kamp H. 2013 d]).

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay. A strong bacteriotoxic effect (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants, reduction in the titer) was observed in the standard plate test depending on the strain and test conditions from about 100 µg/plate onward. In the pre-incubation assay strong bacteriotoxicity (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants, reduction in the titer) was observed depending on the strain and test conditions from about 33 µg/plate onward.

C. MUTATION ASSAYS

Neither in the original nor in the confirmatory experiment with and without metabolic activation a biologically relevant increase in number of revertants was observed in any strain tested (see Table 5.8.1-27). The negative and positive controls yielded revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system. No test substance precipitation was found with and without S9 mix.

Table 5.8.1-27: Bacterial gene mutation assay with Reg. No. 89517 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Experiment 1: Plate incorporation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	28	18	54	36	15	12	7	6	73	74
Reg. No. 89517										
33 µg/plate	24	19	47	34	14	14	7	6	74	73
100 µg/plate	25	15	42	50	15	11	8	7	74	65
333 µg/plate	20	15	28	24	6	10	6	5	49	44
1000 µg/plate	7	0B	6	0B	5	0B	2	0B	12	8
2500 µg/plate	0B	0B	0B	0B	0B	0B	0B	0B	0B	0B
5000 µg/plate	0B	0B	0B	0B	0B	0B	0B	0B	0B	0B
Pos. control§	968	608	1295	1068	426	1006	271	413	384	795
Experiment 2: Plate incorporation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg.control (DMSO)	-	23	-	41	-	13	-	8	-	-
Reg. No. 89517										
1 µg/plate	-	24	-	41	-	14	-	8	-	-
3.3 µg/plate	-	22	-	41	-	11	-	8	-	-
10 µg/plate	-	21	-	40	-	10	-	8	-	-
33 µg/plate	-	22	-	42	-	13	-	7	-	-
100 µg/plate	-	14	-	38	-	12	-	7	-	-
333 µg/plate	-	11	-	24	-	7	-	2	-	-
Pos. control§	-	760	-	1624	-	1480	-	660	-	-
Experiment 3: Pre-incubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg.control (DMSO)	25	18	38	35	12	11	7	7	37	39
Reg. No. 89517										
1 µg/plate	22	18	39	36	11	13	7	7	35	38
3.3 µg/plate	26	17	39	38	12	13	8	6	35	33
10 µg/plate	28	19	37	35	11	9	9	6	36	40
33 µg/plate	25	11	38	36	14	10	8	7	35	37
100 µg/plate	23	8	44	39	10	10	6	3	34	28
333 µg/plate	15	0B	27	16	8	0B	6	0B	18	20
Pos. control§	846	749	1372	1046	360	967	219	519	349	837

§ = Compound and concentrations see Material and Methods (I.A.2.) above
B = reduced background growth

III. CONCLUSIONS

According to the results of the present study, the test substance Reg. No. 89517 is <u>not</u> mutagenic in the Salmonella typhimurium/Escherichia coli reverse mutation assay under the experimental conditions chosen here.

Report:	CA 5.8.1/14 Schulz M.,Landsiedel R., 2013a Reg.No. 89517 (metabolite of BAS 610 F, Iprodione) - In vitro micronucleus assay in V79 cells (cytokinesis block method) 2013/1286160
Guidelines:	Commission Regulation EU No. 640/2012 of 06 July 2012 - B.49: In vitro Mammalian Cell Micronucleus Test, OECD 487 (2010)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Reg. No. 89517 (Batch OB0002; purity 99.9%) was tested in vitro for its potential to induce micronuclei in V79 cells of the Chinese hamster in the absence and presence of metabolic activation by S9 mix in two independent experiments. Concentrations were chosen based on the results of a pre-test with 16 to 2050 µg/mL. Two independent experiments were performed. In Experiment I (4.89-150 µg/mL), the exposure period was 4 hours with and without S9 mix. In Experiment II, the exposure periods were 4 hours with S9 mix (6.25-200 µg/mL) and 24 hours without S9 mix (1.56-50 µg/mL). The cells were prepared 24 hours after start of treatment with the test item, except for the 4 hour treatment interval in the presence of metabolic activation (Experiment II). In this experimental part cells were harvested after 44 hours to consider possibly occurring mitotic delay. Cytotoxicity indicated by clearly reduced relative increase in cell count (RICC), proliferation index (CBPI) or replicative index (RI) was observed at least at the highest applied test substance concentration in all experimental parts of this study. In both experiments with and without metabolic activation, test substance precipitation in culture medium at the end of exposure period was macroscopically observed from 100 µg/mL onward. No statistically significant or biologically relevant increase in the number of micronucleated cells was observed either without S9 mix or after the addition of a metabolizing system. Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test. Based on the results of this study, Reg. No. 89517 is considered not to induce micronuclei in vitro in V79 cells in the presence or absence of metabolic activation.

(BASF DocID 2013/1286160)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Reg. No. 89517 (Metabolite of BAS 610 F, iprodione)
Description:	Solid, white
Lot/Batch #:	OB0002
Purity:	99.9 % (tolerance ± 1.0 %)
Stability of test compound:	The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 Nov 2019 as indicated by the sponsor, and the sponsor holds this responsibility.
Solvent used:	Dimethylsulfoxide (DMSO)
2. Control Materials:	
Negative control:	A negative control was not employed in this study
Solvent control:	DMSO
Positive controls, -S9:	Ethyl methansulfonate (EMS, 300 and 400 µg/mL, dissolved in MEM without FCS)
Positive control, +S9:	Cyclophosphamide (CPA, 0.5 and 1.0 µg/mL, dissolved in MEM without FCS)

3. Activation:

S9 was produced from male Wistar rats. The rats were induced by intraperitoneal applications of 80 mg/kg bw phenobarbital and by oral administrations of 80 mg/kg bw β-naphthoflavone each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g. Aliquots of the supernatant were frozen and stored at -70 to -80 °C. The S9 mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction was thawed at room temperature and 1 volume of S9-fraction was 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

4. Test organisms:

Chinese hamster V79 cells

This is a continuous cell line with a high proliferation doubling time of 12-14 hours, a high plating efficiency (≥ 90%) and a stable karyotype (modal number of 22 chromosomes).

5. Culture medium/conditions:

Culture media:	Minimal essential medium with Earle's salts (MEM) containing a L-glutamine source supplemented with 10% (v/v) fetal calf serum (FCS), 1% (v/v) penicillin/streptomycin (10 000 IU / 10 000 µg/mL) and 1% (v/v) amphotericine B (250 µg/mL). During exposure to the test substance in the presence of S9 mix MEM medium was used without FCS supplementation.
Cell culture:	Deep-frozen cell stocks were thawed at 37°C in a water bath, and volumes of 0.5 mL were transferred into 25 cm² plastic flasks containing about 5 mL MEM supplemented with FCS. Cells were grown with 5% (v/v) CO_2 at 37°C and ≥ 90% humidity and subcultured twice weekly. Cell monolayers were suspended in culture medium after detachment with 0.25% (w/v) trypsin solution.
Cell cycle and harvest time:	The cell cycle of the untreated V79 cells lasts for about 12 - 14 hours under the selected culture conditions. Thus, a harvest time of 24 hours is about 2 times the normal cell cycle length. V79 cells are an asynchronous cell population, i.e. at the time of test substance treatment there are different cell stages. Since the effect on these cell stages may vary for different test substances, more than one harvest time after treatment may be appropriate. Furthermore, substance-induced mitotic delay may considerably delay the first post-treatment mitosis. Therefore, delayed harvest times (e.g. 44 hours) and prolonged exposure periods (e.g. 24 hours treatment) were considered.

6. Test concentrations:

a) Preliminary toxicity assay: 16. - 2050.0 µg/mL with and without metabolic activation

Exposure period: 4 hours with and without metabolic activation; 24 hours without metabolic activation

Harvest time: 24 hours

b) Cytogenicity assay: 4.89 - 150.0 µg/mL with and without metabolic activation

6.25 - 200.0 µg/mL with metabolic activation

1.56 - 50.0 µg/mL without metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 22-Jul-2013 - 18-Nov-2013

2. Preliminary cytotoxicity assay: In the pretest for toxicity based on the purity and the molecular weight of the test substance 2050 µg/mL (approx. 10 mM) of Reg. No. 89517 was used as top concentration. The pretest was performed following the method described for the main experiment. As indication of test substance toxicity relative increase in cell count (RICC) and cell attachment (morphology) were determined for dose selection. PH and solubility were additionally determined.

3. Cytogenicity Assay:

Seeding of cells / treatment: Routinely grown cells that reached a confluency of at least 50% and a max. of 15 passages, were detached by trypsination and used to prepare a single cell suspension with the required cell count (3 - 5x10^5 cells per culture, depending on the schedule) in MEM incl. 10% (v/v) FCS. 5 mL cell suspension were transferred into 25 cm² cell culture flasks using a dispenser. Subsequently, the test cultures were incubated. After an attachment period of about 20 - 24 hours, the medium was removed from the flasks and the treatment medium was added. In case of experiments without metabolic activation the treatment medium consisted of 5 or 4 mL MEM medium with FCS plus 1 mL positive control or 0.05 mL test substance preparation/vehicle, respectively. In case of metabolic activation the treatment medium consisted of 4 or 3 mL Ham's F12 medium without FCS, 1 mL positive control or 0.05 mL test substance preparation/vehicle and 1 mL S9-mix, respectively. The cultures were incubated for the respective exposure period at 37°C, 5% (v/v) CO_2 and ≥ 90% humidity.

At the end of the exposure period, the medium was removed and the cultures were rinsed twice with 5 mL HBSS (Hanks Balanced Salt Solution). Subsequently, 5 mL MEM incl. 10% FCS supplemented with Cytochalasin B (CytB, final concentration: 3 µg/mL; stock: 0.6 mg/mL in DMSO) was added and incubated for the respective recovery time. In the case of 24-hour continuous exposure, CytB was added to the treatment medium at start of treatment, and cell preparation was started directly at the end of exposure. At 44 hours preparation interval in the presence of S9 mix the supplementation of CytB was 24 hours before preparation of the cultures.

Cell harvest, preparation of Slides and staining:	Just before preparation the culture medium was completely removed. Single cell suspensions were prepared from each test group by trypsination. Then, the cell numbers per flask of each single cell suspension were determined using a cell counter. Subsequently, $5x10^4$ cells per slide were centrifuged at 1400 rpm for 7 minutes onto labelled slides using a Cytospin centrifuge. After drying, the slides were fixed in 90% (v/v) methanol for 10 minutes. Before scoring, the slides were stained with a mixture of 4',6-diamideino-2-phenylindole dihydrochloride (DAPI) and propidium iodide (PI) in Fluoroshield™ at a concentration of 0.25 µg/mL each. By the use of the combination of both fluorescence dyes it can be differentiated between DNA (DAPI; excitation: 350 nm, emission: 460 nm) and cytoplasm (PI; excitation: 488 nm, emission: 590 nm).
Analysis of micronuclei and cytotoxicity:	Cytospin slides were scored by fluorescence microscopy. At least 1000 cells per culture, means at least 2000 cells per test group, were evaluated and the number of micronuclei-containing binucleated cells was recorded. Analysis of micronuclei was carried out following the criteria: The diameter of the micronucleus is less than 1/3 of the main nucleus; the micronucleus and main nucleus retain the same color; the micronucleus is not linked to the main nucleus and is located within the cytoplasm of the cell; only cells clearly surrounded by a nuclear membrane were scored. Cultures with few isolated cells were not analyzed for micronuclei.

Relative increase in cell count (RICC):

$$RICC = \frac{(\text{Increase in number of cells in treated cultures (final - starting)})}{(\text{Increase in number of cells in control cultures (final - staring)})} x100$$

Thus, a RICC of 53% indicates 47% cytotoxicity/cytostasis.

Proliferation Index (CBPI):

$$CBPI = \frac{((\text{No. mononucleate cells}) + (2x\text{No. binucleate cells}) + (3x\text{No. multinucleate cells}))}{(\text{Totoal number of cells})}$$

The CBPI was used to calculate the % cytostasis (relative inhibition of cell growth compared to the respective vehicle control group) - a CBPI of 1 (all cells are mononucleate) is equivalent to 100% cytostasis.

% Cytostasis = 100 - 100 $\{(CBPI_T - 1) / (CBPI_C - 1)\}$

(T = test substance treated culture; C = vehicle control culture)

Replicative index (RI):

$$RI = \frac{(((\text{No. binucleate cells}) + (2x\text{multinucleate cells}) \div (\text{Total number of cells}))_T}{(((\text{No. binucleate cells}) + (2x\text{multinucleate cells}) \div (\text{Total number of cells}))_C} x100$$

(T = test substance treated culture; C = vehicle control culture)

Thus, an RI of 53% means that in comparison to the respective control cultures only 53% of the cells divided in the treated test group (= 47% cytotoxicity/cytostasis).

4. Statistics:

A comparison of each dose group with the concurrent vehicle control group at the five and one percent level was carried out using Fisher's exact test for the hypothesis of equal proportions. This test is Bonferroni-Holm corrected versus the dose groups separately for each time and was performed one-sided.

5. Evaluation criteria:

A test item was classified as mutagenic if:

- A significant, dose-related and reproducible increase in the number of cells containing micronuclei was observed.
- The number of micronucleated cells exceeded both the value of the concurrent vehicle control and the range of the laboratory's recent negative control data.

A test item can be classified as non-mutagenic if:

- The number of micronucleated cells in the test groups is not distinctly increased above the concurrent vehicle control and is within the laboratory's recent 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 was verified analytically ([see 2014/1095065 Becker M.,Kamp H. 2013 d]).

B. PRELIMINARY CYTOTOXICITY ASSAY:

In the pre-test cytotoxicity indicated by reduced RICC of about or below 40 - 50% was observed at 64.1 µg/mL and above after 4 hours treatment in the absence of S9 mix. In addition, in the presence of S9 mix, clearly reduced relative increase in cell count was observed after treatment with 128.1 µg/mL and above.

C. CYTOGENICITY ASSAYS:

Two independent experiments were performed. In Experiment I, the exposure period was 4 hours with and without S9 mix. In Experiment II, the exposure periods were 4 hours with S9 mix and 24 hours without S9 mix. The cells were prepared 24 hours after start of treatment with the test item, except for the 4 hour treatment interval in the presence of metabolic activation (Experiment II). In this experimental part cells were harvested after 44 hours to consider possibly occurring mitotic delay. Cytotoxicity indicated by clearly reduced relative increase in cell count (RICC), proliferation index (CBPI) or replicative index (RI) was observed at least at the highest applied test substance concentration in all experimental parts of this study. In both experiments with and without metabolic activation, test substance precipitation in culture medium at the end of exposure period was macroscopically observed from 100 µg/mL onward. In this study, no statistically significant or biologically relevant increase in the number of micronucleated cells was observed either without S9 mix or after the addition of a metabolizing system. In both experiments in the absence and presence of metabolic activation after 4 and 24 hours treatment with the test substance the values (0.2 – 0.7% micronucleated cells) were close to the concurrent vehicle control values (0.3 – 0.7% micronucleated cells) and clearly within our historical negative control data range (0.1 - 1.8% micronucleated cells) (see Table 5.8.1-28/Table 5.8.1-29). The positive control substances EMS (without S9 mix; 400 µg/mL) and CPP (with S9 mix; 0.5 or 1.0 µg/mL) induced statistically significant increased micronucleus frequencies.

Table 5.8.1-28: Summary of results of micronucleus test with Reg. No. 89517 (Metabolite of BAS 610 F, iprodione)

					Genotoxicity	Cytotoxicity		
Exp.	Exposure/ Preparation interval	Test groups	S9 mix	Prec.*	Micronucleated cells** [%]	Proliferation index cytostasis (CBPI) [%]	Replicative index (RI) [%]	Relative increase in cell count (RICC) [%]
1	4/24 hrs	Vehicle Control[1]	-	n.d.	0.6	0.0	100.0	100.0
		4.89 µg/mL	-	-	n.d.	n.d.	n.d.	102.6
		9.38 µg/mL	-	-	n.d.	n.d.	n.d.	85.4
		18.75 µg/mL	-	-	0.5	0.7	99.3	104.2
		37.50 µg/mL	-	-	0.7	-1.2	101.2	91.4
		75.00 µg/mL	-	-	0.5	8.2	91.8	41.4
		150.00 µg/mL	-	+	n.e	n.e.	n.e.	-37.4
		Positive control[2]	-	n.d.	0.6	3.7	96.3	86.5
		Positive control[3]	-	n.d.	2.9[S]	3.2	96.8	92.7
2	4/24 hrs	Vehicle Control[1]	-	n.d.	0.3	0.0	100.0	100.0
		1.56 µg/mL	-	-	n.d.	n.d.	n.d.	99.6
		3.13 µg/mL	-	-	n.d.	n.d.	n.d.	100.8
		6.25 µg/mL	-	-	0.2	1.2	98.8	92.2
		12.50 µg/mL	-	-	0.2	19.1	80.9	101.4
		25.00 µg/mL	-	-	0.3	56.3	43.7	114.3
		50.00 µg/mL	-	-	n.e.	n.e.	n.e.	0.5
		Positive control[2]	-	n.d.	1.2[S]	4.0	96.0	116.2
		Positive control[3]	-	n.d.	2.7[S]	4.3	95.7	136.8

* Precipitation in culture medium at the end of exposure period (macroscopic)
** Relative number of binucleated cells with micronuclei per 2000 cells scored per test group
S Frequency statistically significant higher than corresponding control values
n.d. Not determined
n.e. Not evaluated due to strong toxicity
1 DMSO 1 % (v/v)
2 EMS 300 µg/mL
3 EMS 400 µg/mL

Table 5.8.1-29: Summary of results of micronucleus test with Reg. No. 89517 (Metabolite of BAS 610 F, iprodione)

					Genotoxicity	Cytotoxicity		
Exp.	Exposure/ Preparation interval	Test groups	S9 mix	Prec.*	Micronucleated cells** [%]	Proliferation index cytostasis (CBPI) [%]	Replicative index (RI) [%]	Relative increase in cell count (RICC) [%]
1	4/24 hrs	Vehicle Control[1]	+	n.d.	0.3	0.0	100.0	100.0
		4.89 µg/mL	+	-	n.d.	n.d.	n.d.	94.2
		9.38 µg/mL	+	-	n.d.	n.d.	n.d.	86.8
		18.75 µg/mL	+	-	0.6	4.0	96.0	79.8
		37.50 µg/mL	+	-	0.5	2.6	97.4	82.3
		75.00 µg/mL	+	-	0.4	4.7	95.3	45.1
		150.00 µg/mL	+	+	n.e.	n.e.	n.e.	-7.2
		Positive control[2]	+	n.d.	7.4[S]	56.6	43.4	45.0
2	4/44 hrs	Vehicle Control[1]	+	n.d.	0.7	0.0	100.0	100.0
		6.25 µg/mL	+	-	n.d.	n.d.	n.d.	129.3
		12.50 µg/mL	+	-	n.d.	n.d.	n.d.	136.3
		25.00 µg/mL	+	-	0.3	2.8	97.2	113.2
		50.00 µg/mL	+	-	0.5	-5.3	105.3	89.7
		100.00 µg/mL	+	+	0.6	8.3	91.7	60.0
		200.00 µg/mL	+	+	n.e.	n.e.	n.e.	-28.5
		Positive control[3]	+	n.d.	6.0[S]	-4.5	104.5	67.8

* Precipitation in culture medium at the end of exposure period (macroscopic)
** Relative number of binucleated cells with micronuclei per 2000 cells scored per test group
S Frequency statistically significant higher than corresponding control values
n.d. Not determined
n.e. Not evaluated due to strong toxicity
1 DMSO 1 % (v/v)
2 CPP 1.0 µg/mL
3 CPP 0.5 µg/mL

III. CONCLUSIONS

Based on the results of the study, Reg. No. 89517 is considered not to have a chromosome-damaging (clastogenic) effect nor to induce numerical chromosomal aberrations (aneugenic activity) under in vitro conditions in V79 cells in the absence and the presence of metabolic activation.

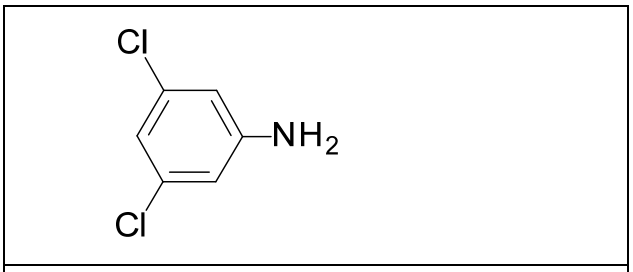
Assessment of genotoxicity for RP44247

RP44247 does not cause point mutations in bacterial cells nor does it induce micronuclei nor point mutations in eukaryotic cells *in vitro*. Overall there is no evidence that RP44247 is genotoxic *in vitro*.

Toxicological evaluation of metabolite RP44247

QSAR analysis predicted a limited alert for genotoxicity *in vitro* with *in silico* metabolic activation which is considered of no relevance. There is no evidence that RP44247 is genotoxic *in vitro.* **In conclusion, RP44247 is considered to be of no genotoxicological concern. Therefore the human health based reference value of 1.5µg/kg bw/d according to the TTC concept for compounds of Cramer Class III can be applied.**

Metabolite RP32596 Reg.No. 85831 (3,5-Dichloroaniline)



RP32596; Reg.No.85831; 3,5-Dichloroaniline

3,5-Dichloroaniline is a metabolite of parent iprodione determined in plant, hydrolysis and soil. Although strucutural similiarty is given to all dioxoimidazolidine-opened metabolites, most apparent to group 2C, 3,5-dichloroaniline does not occur in the rat and was therefore placed into a single metabolite group (3) as proposed by RMS. As there might be potential human consumer exposure via the food chain this metabolite was considered for toxicological relevance assessment.

Structural Alert for 3,5-Dichloroaniline

OASIS-Times gave a negative prediction of genotoxicity for parent metabolite 3,5-dichloroaniline but an in domain positive prediction after *in silico* metabolism for Phenyleneamine. This hypothetical occurring mutagenic derivative potentially emerges by para-oxidation of the dichlorophenyl-ring. However, total predictivity was out of domain mutagen.
CAESAR predicted 3,5-dichloroaniline as non-mutagen in the applicability domain whereas SarPy predicted non-mutagen potentially out of the applicability domain.

In conclusion in one of the structure activity evaluation tools employed there was a limited alert for genotoxicity *in vitro* after *in silico* metabolic activation which is considered of no relevance due to available genotoxicity studies with 3,5-dichloroaniline indicating non-mutagenicity *in vitro* and *in vivo*.

Is is important to note, that for a number of metabolites of iprodione QSAR analysis generated mutagenic alerts after *in silico* activation. The alerting structures are C-nitroso compounds or Quinoneimines which are closely related to 3,5-dichloroaniline (oxidized form of 3,5-dichloroaniline is a C-nitroso compound; reduced form of Phenyleneamine, a predicted metabolite of 3,5-dichloroaniline is a Quinoneimine). The gentoxocity studies performed with 3,5-dichloroaniline proof the non-relevance of these QSAR-based predictions.

Genotoxicity studies for 3,5-Dichloroaniline

Report: CA 5.8.1/15
Engelhardt G., Hoffmann H.D., 1997a
Salmonella typhimurium reverse mutation assay with Norharman - Two screening studies for the comparison of 3,5-Dichloroaniline and 4-Chloroaniline
1997/1002678
Guidelines: OECD 471
GLP: no

Executive Summary

S. typhimurium strain 98 was exposed to 3,5-Dichloroaniline (Batch: L97/332; Purity: 99.8%) using DMSO as a solvent in the presence and absence of metabolic activation both with and without addition of norharman in the standard plate test. Some chemicals like the aromatic amines aniline and o-toluidine show mutagenic activity only after the addition of the tryptophan pyrolysate norharman. Therefore in this study a test was included using also norharman. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment. 3,5-Dichloroaniline was tested at concentrations of 20, 100, 500, 2500 and 5000 µg/plate. A bacteriotoxic effect was observed from about 2500 µg/plate onwards (with and without metabolic activation) but not after adding norharman. A biologically relevant increase in the number of revertant colonies was not noticed in absence of S9 or after metabolic activation both with and without addition of norharman. 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 3,5-Dichloroaniline is not mutagenic in the Salmonella typhimurium strain TA98 under the experimental conditions of the study.

(BASF DocID 1997/1002678)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material 3,5-Dichloroaniline

Description: white crystals

Lot/Batch #: L97/332

Purity: 99.8%

Stability of test compound: No data given in the protocol.

Solvent used: 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 compound tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate

Positive control compound tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate

Positive control compound tested with addition of metabolic activation system and norharman:

Strain	Mutagen	Solvent	Concentration
TA 98	aniline	DMSO	100 µg/plate
TA 98	o-toluidine	DMSO	100 µg/plate

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. 5 days after the administration the animals were sacrificed. 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

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). Histidine auxotrophy is automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

Plate incorporation assay:

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 condition (i.e. with and without S9) for tester strain TA98.

B. TEST PERFORMANCE:

1. Dates of experimental work:
no stated in the report

2. Plate incorporation assay:
To test tubes containing 2-mL portions of warm soft agar 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. In the tests with S9 mix also 0.1 mL norharman solution was added in the tests with norharman. After mixing, the samples are poured onto Vogel-Bonner agar plates (minimal glucose agar plates).
After incubation in the dark for 48 hours at 37°C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

3. Statistics:
No special statistical tests were performed.

4. 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

No information given in the report.

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^- background; decrease in the number of his^+ revertants) was observed in the standard plate test from 2500 µg/plate onwards in the test with and without metabolic activation but not after adding norharman.

C. MUTATION ASSAYS

In the plate incorporation experiments with and without metabolic activation and with or without norharman was no biologically relevant increase in number of revertants observed in strain TA98 (Table 5.8.1-30).

No test substance precipitation was found.

Table 5.8.1-30: Bacterial gene mutation assay with 3,5-Dichloroaniline - Mean number of revertants

Strain	TA 98		
Metabol. Activation	+S9	-S9	+S9 +norharman
Neg. control (DMSO) 5916256	33	25	36
20 µg/plate	37	22	35
100 µg/plate	21	23	31
500 µg/plate	20	14	30
2500 µg/plate	5B	0B	25
5000 µg/plate	0B	0B	28
Pos. control[§]	1036	699	285 (aniline) 305 (o-toluidine)

[§]: Compound and concentrations see Material and Methods (I.A.2.) above
B: Reduced background growth

III. CONCLUSION

According to the results of the present study, the test substance 3,5-Dichloroaniline is not mutagenic in the Salmonella typhimurium reverse mutation assay under the experimental conditions chosen here.

Report:	CA 5.8.1/16 [redacted]., 2013a Reg.No. 85831 (metabolite of BAS 610 F, Iprodione) - Micronucleus assay on bone marrow cells of the mouse - Oral administration 2013/1140617
Guidelines:	OECD 474 (1997), (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.12, EPA 870.5395
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

Reg. No. 85831 (batch: 29027/8, purity: 99.1%) was tested for the potential to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of NMRI mice. For this purpose, the test substance suspended in corn oil was administered once orally to groups of 7 male mice at dose levels of 112.5, 225, and 450 mg/kg body weight in a volume of 10 mL/kg body weight. 5 animals each were administered corn oil as vehicle and cyclophosphamide as positive control. The animals were sacrificed 24 hours after the administration. Additional 5 vehicle and 7 high dose group animals were sacrificed 48 hours after treatment. Bone marrow of the femora was prepared. After staining of the cell preparations, 2000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. To investigate a cytotoxic effect due to the treatment with the test item, the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and reported as the number of PCEs per 2000 erythrocytes. Oral administration of Reg. No. 85831 did not lead to any biologically relevant increase in the number of polychromatic erythrocytes containing micronuclei. The rate of micronuclei was under or 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. Signs of systemic toxicity were observed in all treatment groups and consisted of reduction of spontaneous activity, abdominal/hunched posture, eyelid closur, ruffled fur, tumbling, apathy and darker stained eyes (Cyanosis). 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. Based on the results of this study, Reg. No. 85831 is considered to not induce micronuclei in bone marrow cells of the mouse.

(BASF DocID 2013/1140617)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Reg. No. 85831 (Metabolite of BAS 610 F, iprodione)
Description:	Solid, beige
Lot/Batch #:	29027/8
Purity:	99.1%
Stability of test compound:	Homogeneity and concentration of the test substance in the vehicle was confirmed indirectly by dose formulation analytics ([see 2013/1168537 Schuster E.,Kamp H. 2013 b]).
Solvent used:	Corn oil
2. Control Materials:	
Negative:	Vehicle of the test item.
Solvent control:	Corn oil
Positive control:	Cyclophosphamide (CPA) 40 mg/kg bw
3. Test animals:	
Species:	Mice
Strain:	NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	8 - 11 weeks
Weight at dosing:	Mean value of 36.1 g (SD ± 1.3 g)
Source:	Charles River Laboratories, Research Models and Services Germany GmbH, Sandhofer Weg 7, 97633 Sulzfeld, Germany
Number of animals per dose:	7 (5 in vehicle and positive control group)
Acclimation period:	At least 5 days
Diet:	Pelleted standard diet (Harlan Laboratories B.V.; Postbus 6174; 5960 AD Horst; The Netherlands), ad libitum
Water:	Tap water, ad libitum
Housing:	The animals were housed individually in Makrolon Type II/III, with wire mesh top.

4. Environmental conditions:

Temperature:	22 ± 2 °C
Humidity:	45 - 65%
Air changes:	frequency not indicated
Photo period:	12-hour light-dark cycle

5. Test compound concentration:

Range finding test:	300, 450, 900 mg/kg bw (10 mL/kg bw)
Micronucleus assay:	112.5, 225 and 450 mg/kg bw (10 mL/kg bw)

B. TEST PERFORMANCE

1. Dates of experimental work: 18-Dec-2012 to 28-Feb-2013

2. Preliminary range finding test:

Male and female NMRI mice were administered the test substance once orally at doses of 300, 450 and 900 mg/kg bw.

3. Micronucleus test:

Treatment and sampling: Groups of 7 male mice were treated once with 112.5, 225 or 450 mg/kg bw of the test substance by oral gavage. The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the first day of administration. The vehicle control and the positive control substance CPA were administered once by oral gavage (10 mL/kg bw). The animals were surveyed for evident clinical signs of toxicity throughout the study. Twenty-four hours after the administration the mice were killed using CO_2 followed by bleeding. Additional 5 control group animals and 7 high dose group animals were killed after 48 hours. After sacrifice, the bone marrow was prepared.

Preparation of animals/slides:	The femora were removed, the epiphyses were cut off and the marrow was flushed out with foetal calf serum using a syringe. The cell suspension was centrifuged at 1500 rpm (390 x g) for 10 minutes and the supernatant was discarded. A small drop of the re-suspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald (Merck, 64293 Darmstadt, Germany)/Giemsa (Merck, 64293 Darmstadt, Germany). Cover slips were mounted with EUKITT (Kindler, 79110 Freiburg, Germany). At least one slide was made from each bone marrow sample.
Slide evaluation:	Evaluation of the slides was performed using NIKON microscopes with 100x oil immersion objectives. Per animal at least 2000 polychromatic erythrocytes (PCE) were analysed for micronuclei. To investigate a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 2000 erythrocytes. The analysis was performed with coded slides.

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 as an aid in evaluating the results, if necessary. However, the primary point of consideration was the biological relevance of the results.

5. Evaluation criteria:

The study was considered valid as at least 5 animals per test group could be evaluated, PCE to erythrocyte ratio should not be less than 20% of the vehicle control and the positive control shows a statistically significant and biological relevant increase of micronucleated PCEs compared to the vehicle control. A test item was 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. A test item 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 dose formulation analytics was performed as a separate study by the sponsor and reported in a separate report ([see 2013/1168537 Schuster E.,Kamp H. 2013 b]).
Samples of all suspended dose formulations and of the vehicle control (1 x 1 mL, each) were taken on the first treatment day immediately after the last application of the test item. As the test item could only be suspended in the vehicle, additional samples from top, middle and bottom of all dose formulations (1 x 1 mL, each) were taken. Corresponding reserve samples were also taken and all aliquots were stored at ≤ -18 °C until one set of samples was shipped frozen (on dry ice) for analysis to the sponsor. The second set of samples was stored at ≤ -18 °C at Harlan CCR as reserve samples.
The homogeneity and concentration of the test substance preparation in the vehicle corn oil was verified by HPLC. The mean concentrations were determined as 10.52-11.16, 20.78-22.82, and 42.34-45.82 mg/mL at nominal concentrations of 11.25, 22.5 and 45.0 mg/mL, respectively. This corresponds to a recovery rate ranging between 96.9% to 98.8% and is thus, within the expected range (90-110%).
Plasma analytics 24h after treatment detected, but not fully confirmed in two mouse plasma samples 3,5-dichloroaniline based on ion ratio and retention time measurements ([see 2014/1095062 Becker M.,Landsiedel R. 2014 a]). Due to the low sensitivity of the LC-MS method, mainly caused by the physico-chemical properties of the test substance, only relatively high plasma levels are able to be confirmed (approximately 400 ng/mL in mouse plasma).

B. PRELIMINARY RANGE FINDING TEST

Approximately 20 minutes after application all animals of the 900 mg/kg bw dose group had to be euthanized due to moribund conditions. At 300 and 450 mg/kg bw reduction of spontaneous activity, abdominal posture, eyelid closure, ruffled fur, tumbling and darker stained eyes (Cyanosis) were observed. On the basis of these data, 450 mg/kg bw was the maximum tolerated dose and considered to be suitable as highest dose in the main experiment. There were no distinct differences between male and female animals. Thus, only male animals were used for the main experiment.

C. MICRONUCLEUS ASSAY

Signs of systemic toxicity were observed in all treatment groups and consisted of reduction of spontaneous activity, abdominal/hunched posture, eyelid closure, ruffled fur, tumbling, apathy and darker stained eyes (Cyanosis). Clinical signs were evident until sacrifice and were more pronounced in the high dose group. No signs of systemic toxicity were observed in any of the animals treated with the positive control substance or the vehicle.
After treatment with the test item at 24h and 48h preparation interval, the mean number of PCEs per 2000 erythrocytes was not substantially decreased as compared to the mean value of PCEs of the vehicle control. Only few individual mice in the 24-hour high dose group showed slightly lower numbers of PCEs per 2000 erythrocytes. This indicates that Reg. No. 85831 did in general not induce substantial cytotoxic effects in the bone marrow, however a slight individual effect at 450 mg/kg cannot be excluded. Plasma analytics, isolated from mice 24h post treatment detected 3,5-dichloroaniline in two plasma samples ([see 2014/1095062 Becker M.,Landsiedel R. 2014 a]).
In comparison to the vehicle controls, there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item (see Table 5.8.1-31). The mean values of micronuclei observed after treatment with Reg. No. 85831 (0.064-0.114%) were below or near to the value of the vehicle control group (0.100%) and very well within the historical vehicle control data range. The clastogenic activity of the positive control substance cyclophosphamide was evident by the increase in the number of PCE (1.510%). The positive control thus demonstrated the sensitivity of the test system.

Table 5.8.1-31: Micronucleus test in mice administered Reg. No. 85831 by oral gavage

Treatment	Total scored erythrocytes	PCE/ erythrocytes	Micronucleated cells per total number of PCEs	PCEs with micronuclei (%)
24 h sampling				
Corn oil	10000	5883	10	0.100
Reg. No. 85831				
112.5 mg/kg bw	14000	7632	16	0.114
225.0 mg/kg bw	14000	8140	16	0.114
450.0 mg/kg bw	14000	7273	14	0.100
Positive control				
Cyclophosphamide	10000	5770	151	1.510
48 h sampling				
Corn oil	10000	5601	14	0.140
Reg. No. 85831				
450.0 mg/kg bw	14000	8822	9	0.064

III. CONCLUSIONS

Based on the result of this study Reg. No. 85831 does not induce the formation of micronuclei in polychromatic erythrocytes in the bone marrow cells of mice under the conditions of the study.

Assessment of genotoxicity for 3,5-Dichloroaniline

Overall there is no evidence for *in vitro* or *in vivo* genotoxicity of 3,5-dichloroaniline based on the study results available. The results are supported by studies referenced in the open literature demonstrating a lack of genotoxicity in an Ames test (BASF DocID 1987/1002734) and DNA-Repair assay (BASF DocID 1988/1003296).

Short-term toxicity of 3,5-Dichloroaniline

Report:	CA 5.8.1/17 [redacted]., 2014a Reg.No. 85831 (metabolite of BAS 610 F, Iprodione) - Repeated-dose 28-day toxicity study in Wistar rats - Administration by gavage 2013/1286162
Guidelines:	OECD 407, (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.7 No. L 142, EPA 870.3050
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Reg. No. 85831 (Batch: L80-168; Purity: 99.9%) was administered by gavage to five male and female Wistar rats each at concentrations of 0, 7.5, 20 and 50 mg/kg bw/d for at least 28 days. Clinical pathology revealed a macrocytic, hypochromic anemia which was present in rats of both sexes of the mid and high dose group. The effect was indicated by decreased red blood cell (RBC) counts, hemoglobin, hematocrit and mean corpuscular hemoglobin concentration (MCHC) values and increased mean corpuscular volume (MCV) values. The anemia was regenerative because relative reticulocyte counts were increased. The cause of the anemia was hemolysis followed by an increase of total bilirubin values which was present in rats of both sexes of the mentioned test groups. The spleen was identified as target organ at gross pathology. In the spleen of all high dosed animals, extramedullary hematopoiesis mostly graded moderate was observed. This finding was also present in 4/5 and in 3/5 females of the 20 mg/kg bw/d dose group graded minimal to moderate. Pigment storage in the spleen was graded and confirmed as Fe^{3+} by Perl's stain. The pigment storage in the spleen was interpreted to be a sign of increased destruction of erythrocytes and the increased reticulocytes an indication of regeneration of red blood cells. The severity of the iron deposition in the red pulp was dose-dependently increased in males when compared to controls. Females of the control group showed slight deposition and treated females of the mid and high dose a moderate deposition. These findings correlated to an observed dose-dependent increase in absolute and relative spleen weight in male and female animals. Therefore, under the conditions of the present study and based on the hematological and clinical chemistry changes, spleen weight alterations and adverse spleen histopathology observed in both sexes of the 20 and 50 mg/kg bw/d dose groups, the no observed adverse effect level (NOAEL) for Reg. No. 85831 in male and female rats is 7.5 mg/kg bw/d.

(BASF DocID 2013/1286162)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 85831 (Metabolite of BAS 610 F, iprodione)

Description:	Solid / beige
Lot/Batch #:	L80-168
Purity:	99.9% (tolerance± 1.0 %, study code ASAP12_259)
Stability of test compound:	The stability of the test substance under storage conditions over the test period was guaranteed by the sponsor, and the sponsor holds this responsibility (expiry date: 01 February 2015)

2. Vehicle and/or positive control: drinking water containing 1% carboxymethylcellulose

3. Test animals:

Species:	Rat
Strain:	Crl:WI (Han)
Sex:	Male and female
Age:	42 ± 1 day at start of administration
Weight at dosing start:	♂: 171.9 – 173.6 g ♀: 130.6 – 133.6 g
Source:	Charles River Laboratories, Research Models and Services GmbH, Sulzfeld, Germany
Acclimation period:	12 days
Diet:	Kliba maintenance diet for mouse/rats "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	Tap water in bottles, ad libitum
Housing:	Group housing (5 animals per cage) in polysulfonate cages supplied by TECNIPLAST, Hohenpeißenberg, Germany (floor area about 2065 cm^2) Motor activity measurements were conducted in new clean polycarbonate cages with a small amount of bedding for the duration of the measurement.

Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	15 / 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-Aug-2013 - 13-Dec-2013
(In life dates: 18-Aug-2013 (start of administration) to 16-Sep-2013 (necropsy))

2. Animal assignment and treatment:

Reg. No. 85831 (Metabolite of BAS 610 F, iprodione) was administered orally by gavage to groups of 5 male and 5 female rats at concentrations of 0, 7.5 (low dose), 20 (mid dose) and 50 mg/kg bw/d (high dose) over a period of 28 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, preparation frequency and analyses of the test-substance preparations:

Reg. No. 85831 (Metabolite of BAS 610 F, iprodione) was applied as a suspension. Before preparing the test substance, it was pestled and filtered through a smaller than 250 µm filter. Because of problems with the analytics from the 2nd preparation on, the test substance preparation was expanded with Cremophor (5 mg/100 mL). To prepare this suspension, the appropriate amount of test substance was weighed out depending on the desired concentration. Then, drinking water containing 1% carboxymethylcellulose was filled up to the desired volume, subsequently released with a magnetic stirrer. During administration of the test substance, preparations were kept homogeneous by stirring with a magnetic stirrer. The test substance preparations were produced at least once a week and were stored in a refrigerator.

The stability of Reg. No. 85831 (Metabolite of BAS 610 F, iprodione), in drinking water containing 1 % carboxymethylcellulose over a period of 7 days was demonstrated before the start of the study (see Part III BASF Study No.: 01Y0756/12Y082).

Homogeneity of Reg. No. 85831 (Metabolite of BAS 610 F, iprodione) was verified in the highest and lowest concentration (see Part III (supplement)). Additionally, concentration control was verified in all concentrations at the beginning of the study. Due to analytical problems the analyses were repeated on study day 3 with a modified preparation procedure.

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

Parameters	Statistical test	Markers in the tables
Body weight, body weight change	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means	* for $p \leq 0.05$ ** for $p \leq 0.01$
Feces, rearing, grip strength forelimbs, grip strength hindlimbs, foot-splay test, motor activity	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	* for $p \leq 0.05$ ** for $p \leq 0.01$

Statistics of clinical pathology

Parameter	Statistical test	Markers in the tables
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 For parameters with **unidirectional** changes: Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$
Urinalysis parameters (apart from pH, urine volume, specific gravity, color and turbidity)	Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$
Urine pH, volume, specific gravity, color and turbidity	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. Urine color and turbidity are not evaluated statistically.	* for $p \leq 0.05$ ** for $p \leq 0.01$

Statistics of pathology

Parameter	Statistical test	Markers in the tables
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 WILCOXON-test (two-sided) for the equal medians	* for $p \leq 0.05$ ** for $p \leq$-- 0.01

C. METHODS

1. Observations:

A check for moribund and dead animals was made twice daily on working days and once daily on Saturdays, Sundays and public holidays. If animals were in a moribund state, they were sacrificed and necropsied. All rats were checked daily for any abnormal clinically signs before the administration as well as within 2 hours and within 5 hours after the administration. Detailed clinical observations (DCO) were performed in all animals prior to the administration period and thereafter at weekly intervals. The findings were ranked according to the degree of severity, if applicable. The animals were transferred to a standard arena (50 × 37.5 cm with sides of 25 cm height). The following parameters were examined:

1. abnormal behavior during handling
2. fur
3. skin
4. body posture
5. salivation
6. respiration
7. activity/arousal level
8. tremors
9. convulsions
10. abnormal movements
11. impairment of gait
12. lacrimation
13. palpebral closure
14. exophthalmus
15. feces (appearance/consistency)
16. urine
17. pupil size

2. Body weight:

Body weight was determined before the start of the administration period in order to randomize the animals. During the administration period the body weight was determined on day 0 (start of the administration period) and thereafter at weekly intervals. The difference between the body weight on the respective day of weighing and the body weight on day 0 was calculated as body weight change.

3. Food consumption:

Food consumption was determined weekly over a period of 1 day and calculated as mean food consumption in grams per animal and day.

4. Water consumption:

Individual water consumption was determined weekly and calculated as mean water consumption on grams per animal and daily drinking water consumption was monitored by visual inspection of the water bottles for any changes in volume.

5. Ophthalmoscopy:

Not performed in this study.

6. Functional observation battery (FOB):

A functional observational battery was performed in all animals at the end of the administration period starting at about 10:00 a.m. The FOB started with passive observations without disturbing the animals (home cage observations), followed by removal from the home cage and open field observations in a standard arena. Thereafter, sensorimotor tests and reflex tests were conducted. The findings were ranked according to the degree of severity, if applicable. The observations were performed at random.

During the home cage observation special attention was paid to posture, tremors, convulsions, abnormal movements and impairment of gait.

For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm height) and observed for at least 2 minutes. The following parameters were assessed:

1. behavior when removed from cage	10. respiration
2. fur	11. tremors
3. skin	12. convulsions
4. salivation	13. abnormal movements / stereotypes
5. nose discharge	14. impairment of gait
6. lacrimation	15. activity/arousal level
7. eyes/pupil size	16. feces (number of fecal pellets/appearance/ consistency) within two minutes
8. posture	17. urine (appearance/quantity) within two minutes
9. palpebral closure	18. number of rearings within two minutes

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

1. approach response	8. behavior during "handling"
2. touch response	9. vocalization
3. vision ("visual placing response")	10. pain perception ("tail pinch")
4. pupillary reflex	11. grip strength of forelimbs
5. pinna reflex	12. grip strength of hind limbs
6. audition ("startle response")	13. landing foot-splay test
7. coordination of movements ("righting response")	14. other findings

7. Motor activity measurement:

Motor activity was measured at the same day when the FOB was performed. The examinations were performed using the TSE Labmaster System (supplied by TSE Systems GmbH, Bad Homburg, Germany). For this purpose, the rats were placed in new clean polycarbonate cages with a small amount of bedding for the duration of the measurement. Eighteen beams were allocated per cage. The number of beam interrupts was counted over 12 intervals for 5 minutes per interval. The sequence in which the rats were placed in the cages was selected at random. On account of the time needed to place the rats in the cages, the starting time was "staggered" for each animal. The measurement period began when the 1st beam was interrupted and finished exactly 1 hour later. No food or water was offered to the rats during these measurements and the measurement room was darkened after the transfer of the last rat. The program requires a file name for the measured data to be stored. This name consists of the reference number and a serial number.

8. Hematology and clinical chemistry:

Blood was withdrawn in the morning from fasted, isoflurane anesthetized animals from the retro-orbital plexus. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomized sequence.

The following hematological and clinical chemistry parameters were determined for all animals:

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time
✓	Hemoglobin (Hb)	✓	Differential blood count		
✓	Hematocrit (Hct)	✓	Platelet count (PLT)		
✓	Mean corp. volume (MCV)				
✓	Mean corp. hemoglobin (MCH)				
✓	Mean corp. Hb. conc. (MCHC)				
✓	Reticulocytes (RET)				

Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Bile acids	✓	Aspartate aminotransferase (AST)
✓	Phosphorus (inorganic)	✓	Bilirubin (total)	✓	Alkaline phosphatase (ALP)
✓	Potassium	✓	Cholesterol	✓	γ-glutamyl transpeptidase (γ-GT)
✓	Sodium	✓	Creatinine		
		✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

9. Urinalysis:

For urinalysis the individual animals were transferred to metabolism cages and urine was collected overnight. No food or water was supplied during urine collection. The samples were analyzed in a randomized order.

The following parameters were determined for all animals:

Urinalysis					
	Quantitative parameters:		*Semi quantitative parameters*		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Color and turbidity	✓	Urobilinogen
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

10. Sacrifice and pathology:

The animals were sacrificed by decapitation under Isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The following organs were sampled, weighed and examined histopathologically:

Pathology:

The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: control and top dose).

C	W	H		C	W	H		C	W	H	
✓	✓	#	adrenals	✓	✓	#	kidneys	✓		#	skin
✓		#	aorta	✓		#	lacrymal glands[%]	✓		#	spinal cord (3 levels)[@]
✓		#	bone marrow[§]	✓		#	larynx	✓	✓	✓	spleen
✓	✓	#	brain	✓	✓	✓	liver	✓		#	sternum w. marrow
✓		#	caecum	✓		#	lung	✓		#	stomach (fore- & glandular)
✓		#	colon	✓		#	lymph nodes[#]	✓	✓	#	testes
✓		#	duodenum	✓		#	mammary gland	✓	✓	#	thymus
✓	✓	#	epididymides	✓		#	nose/nasal cavity	✓	✓	#	thyroid/parathyroid
✓		#	esophagus	✓	✓	#	ovaries and oviduct**	✓		#	trachea
✓		#	eyes (with optic nerve)	✓		#	pancreas	✓		#	urinary bladder
✓		#	femur (with joint)	✓		#	pharynx	✓	✓	#	uterus
			gall bladder	✓		#	pituitary gland	✓		#	vagina
✓		✓	gross lesions	✓	✓	#	prostate				
✓		#	Harderian glands	✓		#	rectum				
✓	✓	#	heart	✓		#	salivary glands*		✓		body (anesthetized animals)
✓		#	ileum	✓	✓	#	seminal vesicles				
✓		#	jejunum (w. Payer's plaque)	✓		#	skeletal muscle				

[§] from femur; [#] axillary and mesenteric; [@] cervical, thoracic, lumbar; *mandibular and sublingual, ** oviduct not weighed; [%] extraorbital

The organs or tissues were fixed in 4% formaldehyde. From the liver, each one slice of the Lobus dexter lateralis and the Lobus sinister lateralis were fixed in Carnoy's solution and embedded in paraplast. Perl's Prussian blue stain stain was used to confirm the presence of ferric iron ($Fe^{3+)}$ in the spleen.

The immunorelevant organs and tissues were evaluated according to the following parameters:

Thymus:
• Increased/decreased grade of cortico-medullar ratio (related only to area)
• Increase of starry sky cells
• Changes of cellular density in the cortex
• Changes of cellular density in the medulla
Spleen:
• Changes of the cellularity of PALS, lymphoid follicles, marginal zone, red pulp
• Altered cellular composition of follicles
• Altered number of germinal centers
Lymph nodes (mesenteric and axillar lymph nodes):
• Changes in the cellularity of follicles, interfollicular area, paracortical area, medulla
• Altered cellular composition of paracortex
• Altered number of germinal centers
• Hyperplasia of high endothelial venules
Peyer's patches (of the jejunum):
• Changes of the cellularity of follicles (including mantle zone and germinal centers)
• Changes of the cellularity of interfollicular area
Bone marrow:
• Changes of the cellularity
• Changes of the myeloid/erythropoid ratio

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

See Section B 3. above

B. OBSERVATIONS

1. Clinical signs of toxicity

No clinical signs were observed throughout the study.

2. Mortality

No mortality was observed throughout the study.

3. Ophthalmoscopy

Not performed in this study.

4. FOB and Motor Activity

Neither home cage nor open field observations revealed any indication of treatment-related effects. The same holds true for the sensimotor tests and reflexes. All deviations from "zero values" were equally distributed between treated groups and controls or occurred in single animals only and thus were considered to be incidental.
No statistically significant differences of overall motor activity between control and treated animals were observed.

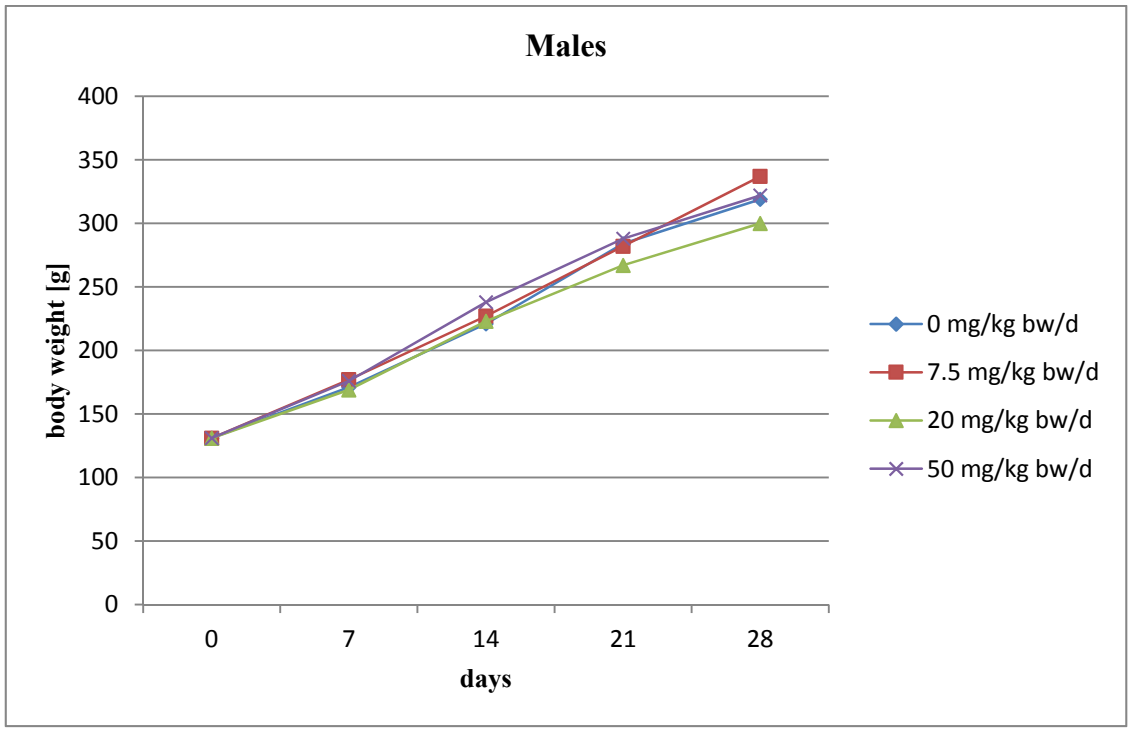
C. BODY WEIGHT AND BODY WEIGHT GAIN

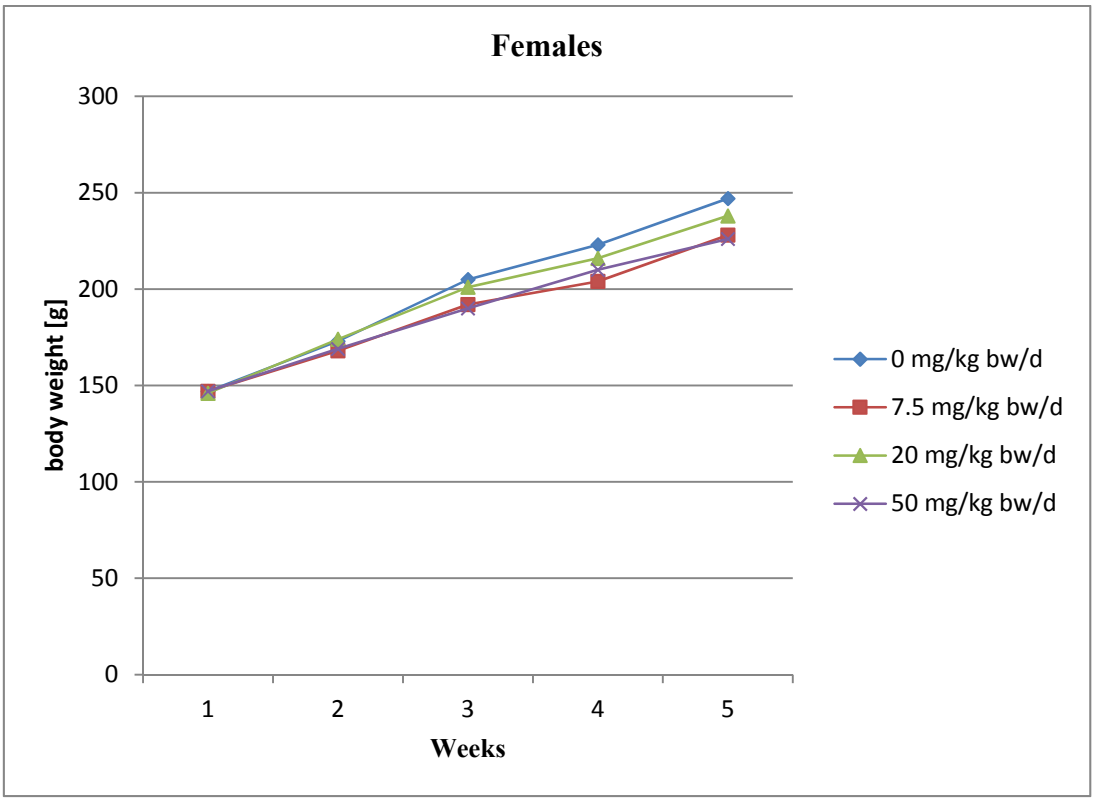
No significant deviations with regard to the mean body weight were noted for male and female animals of all test groups [see Table 5.8.1-32, Figure 5.8.1-2]. The mean value of body weight change in male animals on day 7 was significantly decreased in the 50 mg/kg bw/d dose group. This was a single finding at the beginning of the study and therefore assessed as being incidental.

Table 5.8.1-32: Mean body weight of rats administered Reg. No. 85831 for at least 28 days

	Males				Females			
Dose level [mg/kg bw/d]	0	7.5	20	50	0	7.5	20	50
Body weight [g]								
- Day 0	174.1	171.9	173.5	173.6	131.2	132.3	130.6	133.6
- Day 28	301.2	294.5	293.9	289.0	192.9	190.8	187.6	189.0
Δ% (compared to control)		-2.2	-2.4	-4.1		-1.1	-2.7	-2.0
Overall body weight gain [g]	127.1	122.6	120.5	115.4	61.7	58.4	57.0	55.4
Δ% (compared to control)		-3.5	-5.2	-9.2		-5.3	-7.6	-10.2

Figure 5.8.1-2: Body weight development of rats administered Reg. No. 85831 for at least 28 days





D. FOOD CONSUMPTION AND COMPOUND INTAKE

No test substance-related findings were observed in all test groups.

E. WATER CONSUMPTION

No test substance-related findings were observed in all test groups

F. BLOOD ANALYSIS

1. Hematological findings

At the end of the study, in rats of both sexes of the 50 mg/kg bw/d dose group, red blood cell (RBC) counts, hemoglobin values and mean corpuscular hemoglobin concentration (MCHC) were significantly decreased and mean corpuscular volume (MCV) and relative reticulocyte counts were significantly increased. Hemoglobin levels were already significantly decreased in rats of both sexes of the 20 mg/kg bw/d dose group and relative reticulocyte counts were significantly increased also in this test group. Additionally, in females of the 50 mg/kg bw/d dose group, hematocrit values were significantly decreased and mean corpuscular hemoglobin content (MCH) was significantly increased. In females of the 20 mg/kg bw/d test group, RBC counts and hematocrit values were already decreased and MCV increased. In females treated with 7.5 mg/kg bw/d, hemoglobin values (8.1 mmol/L) were also below those of the controls (8.5 mmol/L), but the mean was within the historical control range (hemoglobin 7.8-9.5 mmol/L). In these individuals mean MCHC (20.91 mmol/L) was also marginally below the historical control range (MCHC 20.99-24.34 mmol/L), but this is a calculated value and the measured parameters (hemoglobin and hematocrit (0.389 L/L)) were within the historical control range (hematocrit 0.349-0.427 L/L). Therefore, the alterations of the red blood cell parameters in females of the 7.5 mg/kg bw/d dose group were regarded as treatment-related but not adverse. In females treated at 7.5 and 50 mg/kg bw/d, platelet counts were significantly increased (843 and 874 giga/L) when compared to the control (746 giga/L), but the increase was not dose-dependent and the means in both test groups were within the historical control range (platelets 779-1060 giga/L). In females of the 20 mg/kg bw/d dose group, relative neutrophil counts were significantly lower (9.6%) when compared to controls (14.3%) whereas relative lymphocyte counts were higher compared to controls (87.0 vs. 81.6%), but both parameters were not dose-dependently changed. Therefore, alterations of platelet counts, relative neutrophil and lymphocyte counts in females of the mentioned test groups were regarded as incidental and not treatment-related.

2. Clinical chemistry findings

At the end of the study in rats of both sexes of the 20 and 50 mg/kg bw/d dose groups total bilirubin levels were increased, reaching statistical significance in animals of the high dose group.

3. Urinalysis

No treatment-related changes among urinalysis parameters were observed

G. NECROPSY

1. Organ weight

When compared to the control group (set to 100%), the mean absolute and relative weights of the spleen were statistically significantly increased in the 20 and 50 mg/kg bw/d dose groups (see Table 5.8.1-33).

Table 5.8.1-33: Selected mean absolute and relative organ weights of rats administered Reg. No. 85831 for at least 28 days

Sex		Males				Females			
Organ weight [mg]	Dose [mg/kg bw/d]	Absolute weight	Δ%	Relative weight	Δ%	Absolute	Δ%	Relative weight	Δ%
Spleen	0	0.532		0.193		0.394		0.22	
	7.5	0.576	(8)	0.211	(9)	0.39	(-1)	0.224	(2)
	20	0.654*	(23)	0.244**	(26)	0.458*	(16)	0.268*	(21)
	50	0.912**	(71)	0.346**	(79)	0.672**	(71)	0.396**	(80)

* $p \leq 0.05$; ** $p \leq 0.01$

No other statistically significant changes of absolute or relative organ weights were observed.

2. Gross and histopathology

At necropsy the following gross lesions were observed: The spleen of one male animal of the 50 mg/kg bw/d dose group showed discoloration and was enlarged. Furthermore, a focus on the adrenal gland observed in one of the test 20 mg/kg bw/d female animals was considered to be incidental or spontaneous in origin and without any relation to treatment.

Extramedullary hematopoiesis and pigment storage in the red pulp of the spleen were observed in males and females of the 20 and 50 mg/kg bw/d dose groups and were considered to be treatment-related. Extramedullary hematopoiesis was accompanied by an increase of hematopoietic cells, especially reticulocytes, in the red pulp of the spleen. Pigment storage was graded and confirmed as Fe^{3+} by Perl's stain: the severity of the iron deposition in the red pulp was increased as compared to controls (see Table 5.8.1-34).

Table 5.8.1-34: Incidence of selected macro- and histopathological lesions in rats administered Reg. No. 85831 for at least 28 days

	Male animals				Female animals			
Test group (mg/kg bw/d)	0	7.5	20	50	0	7.5	20	50
No. of animals	5	5	5	5	5	5	5	5
Hematopoiesis#			4	5			3	5
• Grade 1			4	1				
• Grade 3				4			3	5
Pigment storage§	5	5	5	5	5	5	5	5
• Grade 1	4	5						
• Grade 2	1		4		5	5		
• Grade 3			1	5			5	5

extramedullary
§ Perl´s stain

The following codes were used for a grading system that takes into consideration either the severity or the number or the size of a microscopic finding:

	Severity	Number	Size
Grade 1	Minimal	Very few	Very small
Grade 2	Slight	Few	Small
Grade 3	Moderate	Moderate number	Moderate size
Grade 4	Marked; severe	Many	Large
Grade 5	Massive; extreme	Extensive number	Extensive size

All other findings occurred either individually or were biologically equally distributed over control and treatment groups. They were considered to be incidental or spontaneous in origin and without any relation to treatment.

III. CONCLUSIONS

The administration of Reg.No. 85831 (Metabolite of BAS 610 F, iprodione) by gavage to male and female Wistar rats for 4 weeks induced hematological and clinical chemistry changes, spleen weight alterations and adverse spleen histopathology in both sexes of the 20 and 50 mg/kg bw/d dose groups. No treatment-related findings were observed in animals treated with 7.5 mg/kg bw/d. Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) for this test compound in rats of both sexes is 7.5 mg/kg bw/d.

Assessment of short-term toxicity of 3,5-Dichloroaniline

A no observed adverse effect level (NOAEL) for short-term toxicity was established at 7.5 mg/kg bw/d based on hematological and clinical chemistry changes, spleen weight alterations and adverse spleen histopathology in both sexes of Wistar rats at 20 mg/kg bw/d.

Peer reviewed literatur for 3,5-Dichloroaniline

Report: CA 5.8.1/18
Rankin G.O. et al., 1985a
3,5-Dichloroaniline-induced nephrotoxicity in the Sprague-Dawley rat
1986/1002731
Guidelines: none
GLP: no

Executive Summary of the Literature

The nephrotoxic potential of 3,5-dichloroaniline (DCA, unknown purity) was examined in male Sprague-Dawley rats. Rats were administered DCA as the hydrochlorine salt (0.4, 0.8 or 1.0 mmol/kg, i.p.), or 0.9% saline (1.0 ml/kg, i.p.), and renal function was monitored at 24 and 48h. DCA (0.4 mmol/kg) administration did not produce evidence of nephrotoxicity. However, DCA (0.8 mmol/kg) administration decreased urine volume and osmolality, increased proteinuria, elevated the blood urea nitrogen (BUN) concentration and decreased basal and lactate-stimulated p-aminohippurate (PAH) accumulation. Three of 4 rats receiving DCA (1.0 mmol/kg) died prior to 48h postinjection. Incubation of renal cortical slices with DCA resulted in decreased PAH and tetraethylammonium (TEA) uptake when DCA concentrations of 10^{-6} M or greater were used. These results indicate that DCA is nephrotoxic to Sprague-Dawley rats when administered in a dose of 0.8 mmol/kg or higher and is capable of altering organic ion transport in vitro.

BASF assessment: In an additional study by the same laboratory in 1997 (BASF DocID 1997/1008101) using different chemical forms, application routes and vehicles to assess nephrotoxicity of 3,5-dichloroaniline in the rat it was shown that the hydrochloride salt of 3,5-DCA given i.p. caused effects on the kidney whereas the free base did not, irrespective of the application route (i.p. or orally). In addition it was shown that the hydrochlorid acid at equimolar doses to 3,5-DCA hydrochloride salt was not able to induce nephrotoxicity and concluded that the hydrochloride salt and 3,5-DCA are required to induce nephrotoxicity. 3,5-dichloroaniline as the free base after oral exposure, which is the toxicological relevant form and route of application when considering potential human expsore, does not induce nephrotoxicity in the rat under the conditions of the assay referenced as BASF DocID 1997/1008101. In addition, no evidence for nephrotoxicity was seen in a standard regulatory 28-day study when testing 3,5-DCA up to 50 mg/kg bw/d in male and female Wistar rats (BASF DocID 2013/1286162)

Classification of study: Supplementary information

Report: CA 5.8.1/19
Rashid K.A. et al., 1987a
Mutagenicity of Chloroaniline/Lignin metabolites in the Salmonella/microsome assay
1987/1002734
Guidelines: none
GLP: no

Executive Summary of the Literature

The mutagenic potential of five chloroanilines, including 3,5-dichlororaniline (unknown purity), and their succinamides and succinimide derivatives have been tested wich two strains of Salmonella typhimurium (TA98 and TAl00) with and without rat hepatic microsomal fraction. None of the compounds produced a dose response effect with a two fold increase in revertants indicating that these compounds are not mutagens or promutagens in these assays.

Classification of study: Supplementary information

Report: CA 5.8.1/20
Yoshimi N. et al., 1988a
The genotoxicity of a variety of aniline derivatives in a DNA repair test with primary cultured rat hepatocytes
1988/1003296
Guidelines: none
GLP: no

Executive Summary of the Literature

The genotoxicity of a variety of aniline derivatives was examined by a DNA repair test with rat hepatocytes. 3,5-dichloroaniline (unknown purity) was tested negative under the condition of this assay.

Classification of study: Supplementary information

Report: CA 5.8.1/21
Sabbioni G.,Neumann H.-G., 1990a
Biomonitoring of arylamiinies: Hemoglobin adducts of urea and carbamate pesticides
1990/1004192
Guidelines: none
GLP: no

Executive Summary of the Literature

Hemoglobin adducts of aromatic amines released from pesticides were investigated. Female Wistar rats were dosed orally with pesticides (unknown purity) up to 1 mmol/kg body weight. Blood was obtained after 24 h, hemoglobin isolated and hydrolysed in 1 N NaOH. The amines were extracted and quantified by gas chromatography with nitrogen-specific or mass-selective detection. With vinclozoline and iprodione (3,5-dichloroaniline) no adducts could be found.

Classification of study: Supplementary information

Report: CA 5.8.1/22
Lo H.-H. L. et al., 1990a
Acute nephrotoxicity induced by isomeric dichloroanilines in Fischer 344 rats
1990/1004193
Guidelines: none
GLP: no

Executive Summary of the Literature

The purpose of this study was to examine the nephrotoxic potential of the six dichloroaniline (DCA, unknown purity) isomers in vivo and in vitro. In the in vivo studies, male Fischer 344 rats (4-8 rats/group) were administered a single, intraperitoneal injection of a DCA isomer (0.4, 0.8 or 1.0 mmol/kg) as the hydrochloride salt or given vehicle (0.9% saline, 2.5 ml/kg), and renal function monitored at 24 and 48 h. Renal effects induced by DCA were characterized by decreased urine volume, increased proteinuria, hematuria, modest elevations in blood urea nitrogen (BUN) concentrations, decreased accumulation of p-aminohippurate (PAH) by renal cortical slices, and no change or a slight decrease in kidney weight. Renal morphological changes were observed as proximal tubular necrosis with lesser effects on distal tubular cells and collecting ducts. Based on the overall effects on renal function and morphology, the decreasing order of nephrotoxic potential was found to be 3,5-DCA > 2,5-DCA > 2,4-, 2,6- and 3,4-DCA > 2,3-DCA. The ability for the DCA to induce nephrotoxicity correlated well with the lipophilic properties of the DCA isomers and Hammett constants (σ) for the various chloro substitutions. In the in vitro studies, renal cortical slices from naive male Fischer 344 rats were co-incubated with a DCA isomer (0-10^{-3} M) and PAH or tetraethylammonium (TEA). All DCA isomers decreased PAH and TEA accumulation at 10^{-3} M DCA concentration in the media with 3,5-DCA inducing the largest decrease at this concentration. These results indicate that DCA are capable of altering renal function in vivo and in vitro and that 3,5-DCA possesses the greatest nephrotoxic potential in vivo and in vitro.

BASF assessment: In an additional study by the same laboratory in 1997 (BASF DocID 1997/1008101) using different chemical forms, application routes and vehicles to assess nephrotoxicity of 3,5-dichloroaniline in the rat it was shown that the hydrochloride salt of 3,5-DCA given i.p. caused effects on the kidney whereas the free base did not, irrespective of the application route (i.p. or orally). In addition it was shown that the hydrochlorid acid at equimolar doses to 3,5-DCA hydrochloride salt was not able to induce nephrotoxicity and concluded that the hydrochloride salt and 3,5-DCA are required to induce nephrotoxicity. 3,5-dichloroaniline as the free base after oral exposure, which is the toxicological relevant form and route of application when considering potential human expsore, does not induce nephrotoxicity in the rat under the conditions of the assay referenced as BASF DocID 1997/1008101. In addition, no evidence for nephrotoxicity was seen in a standard regulatory 28-day study when testing 3,5-DCA up to 50 mg/kg bw/d in male and female Wistar rats (BASF DocID 2013/1286162)

Classification of study: Supplementary information

Report: CA 5.8.1/23
Sabbioni G., 1991a
Hemoglobin binding of monocyclic aromatic amines: Molecular dosimetry and quantitative structure activity relationships for the N-oxidation
1992/1005217
Guidelines: none
GLP: no

Executive Summary of the Literature

The authors established the hemoglobin binding index (HBI) [(mmol compound/tool Hb)/(mmol compound/kg body wt)] of several aromatic amines in female Wistar rats. For 3,5-dichloroaniline (purity 97%) the HBI was 0.6 ± 0.1, confirming the essential absence of haemoglobin adduct formation.

Classification of study: Supplementary information

Report: CA 5.8.1/24
Lo H.-H. et al., 1994a
Effect of chemical form, route of administration and vehicle on 3,5-Dichloroaniline-induced nephrotoxicity in the Fischer 344 rat
1994/1005350
Guidelines: none
GLP: no

Executive Summary of the Literature

The purpose of this study was to examine the effect of chemical form, route of administration and vehicle on 3,5-dichloroaniline-induced nephrotoxicity. In one set of studies, male Fischer 344 rats (four to eight per group) were administered a single i.p. injection of 3,5-dichloroaniline free base or hydrochloride salt, cysteine hydrochloride or ornithine hydrochloride (0.8, 1.0 or 1.5 mmol kg^{-1}) (unknown purities) or an appropriate vehicle and renal function monitored for 48 h. Only 3,5-dichloroaniline hydrochloride induced nephrotoxicity that was characterized as acute renal failure. When 3,5-dichloroaniline free base (0.8 mmol kg^{-1}) was administered in dimethyl sulfoxide (DMSO), all rats died within 24 h. In a second experiment, the free base or hydrochloride form of 3,5-dichloroaniline (1.5 mmol kg^{-1}) or vehicle (0.9% saline or sesame oil, respectively) were administered orally and renal function monitored for 48 h. No evidence of nephrotoxicity was observed following either treatment. However, when the hydrochloride salt was given in 25% DMSO in 0.9% saline, all rats died within 24 h, with two rats demonstrating increased proteinuria, glucosuria and hematuria within the first 6 h after treatment. These results demonstrate that 3,5-dichloraniline nephrotoxicity is potentiated by the administration of systemic acid, but that acid alone has no effect on renal function at the dose tested. Also, 3,5-dichloroaniline (hydrochloride or free base form) is less toxic orally than when administered i.p. In addition, when DMSO is used as part of the vehicle, 3,5-dichloroaniline toxicity is potentiated. Thus, chemical form, route of administration and vehicle are all important factors in 3,5-dichloroaniline-induced toxicity.

BASF assessment: 3,5-dichloroaniline as the free base after oral exposure, which is the toxicological relevant form and route of application when considering potential human expsore, does not induce nephrotoxicity in the rat under the conditions of the assay. In addition, no evidence for nephrotoxicity was seen in a standard regulatory 28-day study when testing 3,5-DCA up to 50 mg/kg bw/d in male and female Wistar rats (BASF DocID 2013/1286162)

Classification of study: Supplementary information

Report: CA 5.8.1/25
Valentovic M.A. et al., 1996a
Characterization of methemoglobin formation induced by 3,5-dichloroaniline, 4-amino-2,6-dichlorophenol and 3,5-dichlorophenylhydroxylamine
1997/1008101
Guidelines: none
GLP: no

Executive Summary of the Literature

This study characterized the capacity of 3,5-dichloroaniline (unknown purity) and two putative metabolites to induce methemoglobin formation. In vivo intraperitoneal (i.p.) administration of 0.8 mmol/kg 3,5-dichloroaniline resulted in elevated ($P < 0.05$) methemoglobin levels at 2 and 4 h after injection and returned to control values within 8 h. In vitro methemoglobin generation was monitored in washed erythrocytes incubated for 60 min at 37°C with 4 and 8 mM 3,5-dichloroaniline. Methemoglobin generation in vitro was higher ($P < 0.05$) than control values in erythrocytes incubated for 30 min with 0.2-0.6 mM 4-amino-2,6-dichlorophenol or 5-100mM 3,5-dichlorophenylhydroxylamine. The in vitro methemoglobin generating capacity in decreasing order was: 3,5-dichlorophenylhydroxylamine > 4-amino-2,6-dichlorophenol >> 3,5-dichloroaniline. The results of the in vitro studies further indicated that none of the compounds tested induced lipid peroxidation. Erythrocytes incubated with 5-100µM 3,5-dichlorophenylhydroxylamine in vitro were associated with depletion of glutathione. These results indicated that: (a) 3,5-dichloroaniline and its metabolites can induce methemoglobin formation; (b) the N-hydroxy metabolite was the most potent inducer of hemoglobin oxidation and (c) glutathione depletion was associated with methemoglobin formation by 3,5-dichlorophenylhydroxylamine.

BASF assessment: The potential of 3,5-dichloroaniline to induce haematological changes after oral application, which is the relevant route of exposure for relevance assessment, was adequately assessed in a 28-day study conducted with 3,5-DCA (BASF DocID 2013/1286162)

Classification of study: Supplementary information

Report: CA 5.8.1/26
Hong S.K. et al., 1999a
Haloaniline-induced in vitro nephrotoxicity: Effects of 4-haloanilines and 3,5-dihaloanilines
2000/1024057
Guidelines: none
GLP: no

Executive Summary of the Literature

The purpose of this study was to examine the in vitro nephrotoxic effects of the four 4-haloaniline and four 3,5-dihaloaniline (purity ≥97%) isomers using renal cortical slices obtained from the kidneys of untreated, male Fischer 344 rats. Renal cortical slices were incubated with a haloaniline hydrochloride (0.1, 0.5, 1.0 or 2.0 mM, final concentration) or vehicle for 2 h, and toxicity determined by monitoring lactate dehydrogenase (LDH) release and changes in tissue gluconeogenesis capacity. Among the 3,5-dihaloanilines, 3,5-dibromoaniline proved to be the most potent nephrotoxicant and 3,5-difluoroaniline the least potent nephrotoxicant. LDH release was increased by the dibromo (1.0 and 2.0 mM), dichloro (2.0 mM) and diiodo (2.0 mM) derivatives, but not by 3,5-difluoroaniline.

BASF assessment: In an additional study by the same laboratory in 1997 (BASF DocID 1997/1008101) using different chemical forms, application routes and vehicles to assess nephrotoxicity of 3,5-dichloroaniline in the rat it was shown that the hydrochloride salt of 3,5-DCA given i.p. caused effects on the kidney whereas the free base did not, irrespective of the application route (i.p. or orally). In addition it was shown that the hydrochlorid acid at equimolar doses to 3,5-DCA hydrochloride salt was not able to induce nephrotoxicity and concluded that the hydrochloride salt and 3,5-DCA are required to induce nephrotoxicity. 3,5-dichloroaniline as the free base after oral exposure, which is the toxicological relevant form and route of application when considering potential human expsore, does not induce nephrotoxicity in the rat under the conditions of the assay referenced as BASF DocID 1997/1008101. In addition, no evidence for nephrotoxicity was seen in a standard regulatory 28-day study when testing 3,5-DCA up to 50 mg/kg bw/d in male and female Wistar rats (BASF DocID 2013/1286162)

Classification of study: Supplementary information

BASF Assessment of peer reviewed literatur for 3,5-Dichloroaniline

3,5-Dichloroaniline was tested negative in two *in vitro* genotoxicity assays (Ames, DNA repair assay), did not induce nephrotoxicity in the rat when tested as the free base after oral exposure, which is the toxicological relevant form and route of application when considering potential human expsore and did not form haemoglobin adducts when tested *in vivo* in the rat. Assessment of methemoglobin formation after *in vivo* exposure to the male Fischer rat or *in vitro* to washed erythrocytes indicated methemoglobin formation but not lipid peroxidation, which is adequatelly addressed in a short-term toxicity assay in a standard regulatory study in male and female Wistar rats at doses up to 50mg/kg bw/d (BASF DocID 2013/1286162).

Toxicological evaluation and setting of a human health based reference value for 3,5-dichloroaniline

There is no evidence for genotoxicity of 3,5-dichloroaniline based on appropriate genotoxicity studies *in vitro* and *in vivo*. Toxicity after repeated oral gavage for 28-days of 3,5-dichloroaniline was adequately addressed in a standard regulatory short-term toxicity study in Wistar rats (BASF DocID 2013/1286162). Hematological and clinical chemistry changes, spleen weight alterations and adverse spleen histopathology in both sexes were observed at the LOAEL of 20 mg/kg bw/d. No effects were observed at the NOAEL of 7.5 mg/kg bw/d, the lowest dose tested. Supporting information on the hematotoxic mechanism indicates reversible methemoglobin formation as observed after acute intra peritoneal exposure to 3,5-dichloroaniline in Fischer rats at dose levels comparable to the 28-day study in Wistar rats (0.8 mmol/kg application ≈ 26 mg/kg bw (BASF DocID 1997/1008101)). Therefore, it can be assumed that there is a threshold for the effects on hematology and secondary to the spleen effects which has been adequately established to be above the NOAEL of 7.5 mg/kg bw/d.

Appropriate safety factors to extrapolate from short-term or chronic studies have been proposed and reviewed. For extrapolation from subacute to subchronic studies, ECHA proposes a safety factor of 3 and in addition a safety factor of 2 for subchronic to chronic extrapolation (Guidance on Information Requirements and Chemical Safety Assessment; ECHA-2010-G-19-EN, 2012). Recently, a scientific opinion on the "Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data" was published (EFSA Journal 2012;10(3):2579) supporting the proposed safety factor of 2 for subchronic to chronic extrapolation. For inter- and intra-species variation a total safety factor of 100 is assumed.

The acceptable daily intake (ADI) of 3,5-dichloroaniline, based on the NOAEL of 7.5 mg/kg bw/d is:

ADI = 7500µg/kg bw/d : 2 : 3 : 100 = 12.5 µg/kg bw/d

CA 5.8.2 Supplementary studies on the active substance

Report: CA 5.8.2/1
[redacted], 1998b
Iprodione - Quantification of Iprodione and metabolites in the plasma and testes of the rat following a single oral administration of (14C)-Iprodione
B003653
Guidelines: none
GLP: yes

Executive Summary

Executive Summary

The plasma and testosterone levels of iprodione and/or its metabolites were investigated in male Sprague Dawley rats following the administration of ^{14}C-radiolabelled iprodione. Groups of five rats were gavaged with a single dose of radiolabelled iprodione at a nominal dose level of 70 mg/kg bw. Groups of five rats were sacrificed at 0.5, 1, 2, 4, 6, 10, 24 and 48 hours following dosing, at which point the testes were removed and blood samples taken. Tissues (whole blood, plasma, testes extracts) were analysed for radioactivity using liquid scintillation counting. Tissue radioactivity components (plasma, testes extracts) were further investigated using HPLC. Whole blood radioactivity levels increased rapidly, reaching a maximum level between two and ten hours after administration and decreasing rapidly to 24 hours. The mean ratio of radioactivity in whole blood:plasma of approximately 0.7 was calculated. Levels of radioactivity in the testes paralleled those in the whole blood and plasma samples. The mean ratio of radioactivity in testes:plasma was 0.6. HPLC investigations revealed up to five radioactive peaks in extracts of the testes, the most abundant of which were parent iprodione (up to 8.89 µg/g) and metabolite RP 36115 (up to 8.84 µg/g). Metabolite RP 36112 was also identified at lower levels (up to 1.60 µg/g) in the testes.

I. MATERIAL AND METHODS

A. MATERIALS

1 Test Material:

Description:	iprodione
Lot/Batch no.:	Unlabelled iprodione [Batch TS 1129] Radiolabelled iprodione [Batch TV3015C]
Purity:	Unlabelled iprodione 99.7% purity [^{14}C-U-phenyl]iprodione ≥99% radiopurity Specific activity 2.89 GBq/mmole (78 mCi/mmole)
CAS:	36734-19-7
Stability:	Stability verified over the study period

2 Dosing:

Dose level:	70 mg/kg bw iprodione
Dose vehicle:	0.4% aqueous methylcellulose
Dose volume:	5 mL/kg bw
Dosing period:	1 Day

3 Test animals

Species:	Rat
Strain:	Crl:CD(SD)BR
Sex:	Male
Age:	12-14 weeks
Weight range at dosing:	430-530 g
Source:	Charles River, France
Acclimation period:	6 days
Diet:	Pietrement M20 Pelleted Rodent Diet, *ad libitum* (fasted for 17 hours prior to dosing and one hour following dosing)
Water:	Filtered municipal tap water; *ad libitum*
Housing:	5/cage

4 Environmental conditions:

Cage:	Polycarbonate with microisolator lid
Temperature:	20-24°C
Humidity:	40-60%
Photoperiod:	12/12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

1 Study dates

Study Initiation Date: 27th August 1998
Study Completion Date: 30th October 1998

2 Statistics

Not required, beyond calculation of mean values and associated standard deviations.

II. RESULTS AND DISCUSSION

A DOSE FORMULATION ANALYSIS

Actual concentrations and homogeneity of dose formulations were within acceptable limits.

B TISSUE RADIOACTIVITY LEVELS

Levels of total radioactivity (iprodione and/or its metabolites) in whole blood increased rapidly from 0.5 hours following dosing to reach a maximum at 2-10 hours and subsequently declined, with low levels at 48 hours. Levels of radioactivity in the plasma also increased rapidly from 0.5 hours following dosing to reach a maximum at 2-10 hours and subsequently declined, with low levels at 48 hours. The mean ratio of radioactivity in whole blood:plasma of approximately 0.7 was calculated. Levels of radioactivity in the testes increased rapidly from 0.5 hours following dosing to reach a maximum at 6-10 hours and subsequently declined, with low levels at 48 hours. The mean ratio of radioactivity in testes:plasma was 0.6. Data therefore indicate the rapid absorption, distribution and elimination of iprodione and/or its metabolites following the administration of a single gavage dose.

Table 5.8.2-1: Tissue radioactivity levels

Time	Tissue radioactivity					
	Whole blood		Plasma		Testes	
	µg/g	% dose	µg/g	% dose	µg/g	% dose
0.5h	5.004 ±1.039	0.135 ±0.024	8.111± 2.083	0.070± 0.008	3.337± 0.692	0.038± 0.007
1h	7.340 ±3.491	0.165 ±0.104	12.459± 4.580	0.100± 0.050	7.500± 1.010	0.084± 0.011
2h	12.848 ±2.274	0.406± 0.161	20.944± 2.342	0.238± 0.064	13.032± 1.151	0.134± 0.025
4h	11.991 ±6.354	0.326± 0.095	17.422± 4.924	0.200±0.104	12.494± 3.040	0.132± 0.038
6h	13.457± 3.669	0.468± 0.154	21.753± 6.363	0.259± 0.114	17.484± 6.922	0.194± 0.080
10h	13.700 ±5.462	0.525± 0.259	22.619± 9.928	0.307± 0.163	18.194± 7.635	0.202± 0.096
24h	1.425 ±0.418	0.036± 0.011	1.932± 0.593	0.016± 0.005	0.723± 0.110	0.008± 0.002
48h	0.295 ±0.053	0.009± 0.003	0.320± 0.057	0.004± 0.001	0.088± 0.021	0.001± 0.000

C QUANTIFICATION OF RADIOACTIVE FRACTIONS

Plasma radioactivity peaks eluted from the HPLC column did not correlate with the known retention peaks of iprodione or its metabolites RP 36112 or RP 36115. Further investigations into the nature of the material indicated that the plasma radioactivity consisted of iprodione bound to plasma proteins. Up to five radioactive fractions were shown in testes extracts; the two major eluting peaks were identified as iprodione (tissue levels of up to 8.89 µg/g) and RP 36115 (tissue levels of up to 8.84 µg/g). A further minor peak was identified as RP36112 (tissue levels of up to 1.60 µg/g).

Table 5.8.2-2: Testes radioactive fractions

Time	TMET/1	TMET/2 [RP 36112]	TMET/3 [iprodione]	TMET/4	TMET/5 [RP 36115]
	Tissue concentration (µg/g)				
0.5h	0.08	0.31	1.78	0.01	0.95
1h	0.52	0.53	3.52	0.01	3.25
2h	0.32	0.67	6.77	0.03	5.64
4h	0.21	0.73	5.42	0.08	577
6h	0.06	0.90	6.45	0.14	8.84
10h	0.12	1.60	8.89	0.17	7.79
% administered dose					
0.5h	0.0009	0.0036	0.0209	0.0001	0.0111
1h	0.0057	0.0059	0.0393	0.0001	0.0362
2h	0.0035	0.0073	0.0728	0.0003	0.0606
4h	0.0022	0.0078	0.0576	0.0008	0.0614
6h	0.0007	0.0098	0.0696	0.0015	0.0948
10h	0.0013	0.00176	0.0975	0.0018	0.0855

E DISCUSSION

Administration of a single dose of ^{14}C-radiolabelled iprodione at a dose level of 70 mg/kg bw to male Sprague Dawley rats resulted in a rapid increase in the level of iprodione and/or its metabolites in whole blood, plasma and the testes. Tissue radioactivity levels increased rapidly from 0.5 hours following dosing to reach maxima at 2-10 hours and subsequently decline, with low levels detected at 48 hours following dosing. Radioactivity in the plasma appeared to consist largely of protein-bound iprodione. Up to five radioactive peaks were identified in testes extracts; the major peaks were identified as parent iprodione and the metabolite RP 36115. The metabolite RP 36112 was identified as a minor peak.

III. CONCLUSION

This study demonstrates that iprodione is rapidly absorbed, distributed and eliminated following a single gavage dose of 70 mg/kg bw in the rat. Tissue levels of iprodione and its metabolites in the testes mirrored those in the whole blood and plasma, but were slightly lower than plasma levels. iprodione in the plasma was largely protein-bound; iprodione and its metabolite RP 36115 were identified in the testes at maximum tissue concentrations of 8.89 µg/g and 8.84 µg/g respectively; the metabolite RP 36112 was also identified in the testes at lower concentrations of up to 1.60 µg/g.

Report: CA 5.8.2/2
[redacted]., 2000a
Iprodione - Hepatocellular proliferation and toxicity in the CD-1 mouse following 90 days of continual dietary administration
R014684
Guidelines: none
GLP: yes

Executive Summary

In this study, groups of CD-1 mice (10/sex) were administered iprodione in the diet at concentrations of 0 (controls), 60, 200, 800 or 4000 ppm continuously for 90 days. Five additional mice per sex and group were administered the same dose levels of iprodione for 13 days. Mice were implanted with osmotic mini-pumps containing bromodeoxyuridine (BrdU) seven days before sacrifice. Liver weight was recorded at necropsy and was examined histopathologically. Hepatocyte BrdU incorporation was assessed histochemically. After treatment with iprodione for 13 days, a slight increase in liver weight was apparent in mice of both sexes at the highest dose level of 4000 ppm. Histopathology revealed diffuse centrilobular hepatocyte hypertrophy (graded as slight to mild) in both sexes at the highest dose level. A significant increase in centrilobular hepatocyte proliferation was also seen in both sexes at the highest dose level; similar effects were not seen at lower dose levels or in perilobular hepatocytes. After treatment with iprodione for 90 days, an increase in liver weight was seen in both sexes at the highest dose level of 4000 ppm. Histopathology revealed diffuse centrilobular hepatocyte hypertrophy (graded as slight to moderate) in both sexes at the highest dose level; findings in males were occasionally accompanied by focal hepatocyte necrosis. A significant increase in centrilobular hepatocyte proliferation was also seen in both sexes at the highest dose level; similar effects were not seen at lower dose levels or in perilobular hepatocytes. A NOAEL of 800 ppm (equivalent to mean intakes of 127.1 and 146.9 mg/kg bw/d in males and females respectively) can be determined for this study, based on liver effects at the highest dose level.

I. MATERIAL AND METHODS

A. MATERIALS

1 Test Material:

Description:	iprodione (white granules)
Lot/Batch no.:	9612701
Purity:	981 g/kg
CAS:	36734-19-7
Stability:	Stability verified (seven weeks at <15°C followed by one week at ≈20°C.

2 Dosing:

Dose level:	0, 60, 200, 800, 4000 ppm
Dose vehicle:	Diet
Dose volume:	-
Dosing period:	13 days (5/sex/group) 90 days (10/sex/group)

3 Test animals

Species:	Mouse
Strain:	CD-1
Sex:	Male
Age:	9 weeks at the start of treatment
Weight range at dosing:	26.4-33.6 g (males) 24.4-29.0 g (females)
Source:	Charles River, France
Acclimation period:	7-14 days
Diet:	Certified M20 Powdered Rodent Diet, *ad libitum* (fasted overnight prior to blood sampling)
Water:	Filtered municipal tap water; *ad libitum*
Housing:	5/cage

4 Environmental conditions:

Cage:	Polycarbonate with microisolator lid
Temperature:	20-24°C
Humidity:	40-70%
Photoperiod:	12/12 hours light/dark cycle
Ventilation:	10-15 air changes per hour

B. STUDY DESIGN AND METHODS

1 Study dates

Study Initiation Date: 4th May 1999
Study Completion Date: 28th July 2000

2 Study design

Groups of CD-1 mice were administered iprodione in the diet at concentrations of 0, 60, 200, 800 or 4000 ppm for 13 days (5 mice/sex/group) or 90 days (10 mice/sex/group). All mice were implanted subcutaneously with osmotic mini-pumps containing bromodeoxyuridine (BrdU) seven days before termination. Mice were observed daily for mortality and clinical signs. Bodyweights and food consumption were recorded weekly. Gross necropsy was performed on all animals; weights of the brain and liver were recorded. Histopathology was performed on the liver following standard (haematoxylin and eosin) staining. Cell cycling assessment was carried out using anti-BrdU monoclonal antibodies, a secondary biotinylated antibody, streptavidin-peroxidase complex and the chromogen diaminobenzidine. One thousand hepatocytes were visually scored for BrdU incorporation in liver sections from each mouse. Sections of duodenum were also scored for each mouse, as a measure of staining efficiency.

3 Statistics

Means and standard deviations were calculated for each sex, group and time point. Body weights, food consumption and organ weights were intercompared using Bartlett's test and ANOVA. If Bartlett's test indicated homogeneous variances and the ANOVA was significant, group means were intercompared using Dunnett's test. If Bartlett's test indicated heterogeneous variances, values were compared using the Kruskal-Wallis test. If the Kruskal-Wallis test was significant, the Mann-Whitney test was used. Levels of significance of 0.05 and 0.01 were reported. Cell labelling assay results were analyses using Bartlett's test; if Bartlett's test was not significant, ANOVA was performed. If the ANOVA was significant, group and control means were compared using Dunnett's test. If Bartlett's test indicated heterogeneous variances, values were compared using the Kruskal-Wallis test. If the Kruskal-Wallis test was significant, the Mann-Whitney test was used. Levels of significance of 0.05, 0.01 and 0.001 were reported.

II. RESULTS AND DISCUSSION

A DOSE FORMULATION ANALYSIS

Actual concentrations and homogeneity of dose formulations were within acceptable limits.

B IN LIFE FINDINGS

There were a small number of accidental deaths but no treatment-related mortality. No signs of toxicity were observed in animals administered iprodione for 13 days or for 90 days. Bodyweights and food consumption were unaffected by treatment of mice administered iprodione for 13 days; a slight (but statistically significant) increase in bodyweight was seen in females administered 4000 ppm iprodione for 90 days.

C TERMINAL FINDINGS

In animals treated for 13 days, terminal bodyweights were comparable in all groups. Gross necropsy revealed enlarged liver in the majority of males administered 4000 ppm iprodione. Mean absolute liver weight was significantly higher in females administered 4000 ppm iprodione. Mean relative (to body weight) liver weights were significantly elevated in both sexes at this dose level; mean relative (to brain weight) liver weight was also significantly elevated in females. Histopathological investigation revealed diffuse hypertrophy of the centrilobular hepatocytes (graded as slight or mild according to the laboratory's scale) in all animals at 4000 ppm. Assessment of cell proliferation revealed a marked and statistically significant increase in the proportion of BrdU-positive centrilobular hepatocytes in mice of both sexes administered 4000 ppm iprodione. No effects of treatment were observed at lower dose levels or on perilobular hepatocytes in any treatment group.

In animals treated for 90 days, terminal bodyweights were comparable in all groups. Gross necropsy revealed enlarged and/or dark liver in the majority of mice of both sexes administered 4000 ppm iprodione. Mean absolute and relative (to body weight and bran weight) liver weights were significantly higher in males and females administered 4000 ppm iprodione. Histopathological investigation revealed diffuse hypertrophy of the centrilobular hepatocytes (graded as slight to moderate) according to the laboratory's scale) in all animals at 4000 ppm. An associated slight acute focal hepatocyte necrosis was also observed in the majority of males at 4000 ppm. Assessment of cell proliferation revealed a marked and statistically significant increase in the proportion of BrdU-positive centrilobular hepatocytes in mice of both sexes administered 4000 ppm iprodione. No effects of treatment were observed at lower dose levels or on perilobular hepatocytes in any treatment group.

Table 5.8.2-3: 90-day mouse study: Summary of findings

Dose level (ppm)			**0**	**60**	**200**	**800**	**4000**
Intake (mg/kg bw/d)	**13**	**M**	-	10.3	35.0	137	640
		F	-	10.5	37.4	134	651
	90	**M**	-	9.7	31.3	127	661
		F	-	10.9	36.4	147	678
Enlarged liver (#)	**13**	**M**	-	-	-	-	4/5
		F	-	-	-	-	-
	90	**M**	-	-	-	-	7/9
		F	-	-	-	-	9/10
Dark liver (#)	**13**	**M**	-	-	-	-	-
		F	-	-	-	-	-
	90	**M**	-	-	-	-	8/9
		F	-	-	-	-	7/10
Absolute liver weight (g)	**13**	**M**	1.26±0.114	1.14±0.114	1.18±0.171	1.16 ±0.114	1.44±0.207
		F	1.00±0.071	0.98±0.045	1.06±0.055	0.96 ±0.089	**1.26**±0.055**
	90	**M**	1.24±0.073	1.29±0.110	1.26±0.070	1.30 ±0.125	**1.61**±0.162**
		F	1.02±0.130	1.01±0.110	0.98±0.079	1.09 ±0.088	**1.50**±0.156**
Liver weight (relative to body weight)	**13**	**M**	4.20±0.265	3.98±0.342	4.08±0.465	3.98 ±0.217	**4.94*±0.493**
		F	3.92±0.179	3.94±0.270	4.20±0.122	3.78 ±0.286	**4.98**±0.228**
	90	**M**	3.87±0.324	3.90±0.343	3.89±0.296	3.98 ±0.239	**5.02**±0.338**
		F	3.90±0.350	3.78±0.312	3.74±0.178	4.10 ±0.283	**5.44**±0.310**
Liver weight (relative to brain weight)	**13**	**M**	252.3±26.10	247.8±29.30	238.8±32.30	251.4±34.36	301.3±42.01
		F	219.2±24.82	199.1±15.66	220.6±20.77	204.1±24.10	**269.5**±15.48**
	90	**M**	258.6±9.84	262.7±19.11	259.4±15.41	267.1±24.17	**341.7**±38.53**
		F	212.1±22.00	211.0±15.01	205.9±22.08	226.1±22.33	**327.2**±34.32**
Hepatocyte hypertrophy (#)	**13**	**M**	-	-	-	-	5/5 [1,4,-]
		F	-	-	-	-	5/5 [-,5,-]
	90	**M**	-	-	-	-	9/9 [-,1,8]
		F	-	-	-	-	10/10 [2,7,1]
BrdU positive centrilobular hepatocytes (/1000)	**13**	**M**	4.4±2.93	3.5±2.87	29.9±52.15	13.0 ±13.87	**227.4**±31.59**
		F	11.5±11.00	36.1±54.02	16.3±14.81	12.8 ±12.66	**288.2**±68.04**
	90	**M**	5.9±4.93	8.6 ±6.67	5.7±4.58	8.0 ±5.08	**108.9**±44.40**
		F	14.9±26.52	10.2±3.89	7.8±6.76	19.2 ±17.42	**55.3***±23.21**

**significantly different to controls (p<0.05); **p<0.01; ***p<0.001*
laboratory scale [slight, mild, moderate]

E DISCUSSION

Administration of iprodione at a dietary concentration of 4000 ppm to mice for 13 or 90 days resulted in increased liver weights accompanied by diffuse centrilobular hepatocyte hypertrophy and an increase in BrdU incorporation in centrilobular hepatocytes. No effects were seen at dose levels of 60, 200 or 800 ppm iprodione.

III. CONCLUSION

This study clearly demonstrates that the administration of iprodione to mice at a dietary concentration of 4000 ppm for 13 days (equivalent intakes of 640 and 651 mg/kg bw/d in males and females respectively) or for 90 days (equivalent intakes of 661 and 678 mg/kg bw/d in males and females respectively) results in a proliferative response in centrilobular hepatocytes.

Report: CA 5.8.2/3
Groene E.M. de, 1999b
Human androgen receptor binding assay with the fungicide Iprodione and 7 mammalian metabolites
R014678
Guidelines: none
GLP: yes

Executive Summary

The ability of iprodione and seven iprodione metabolites (RP25040, RP32490, RP36112, RP36114, RP36115, RP36118 and RP36119) to interact with androgen receptors isolated from the T47D human mammary gland carcinoma cell line was assessed in a competitive binding assay utilising a constant concentration of tritiated R1881 (methyltrienolone / Metribolone). The binding affinities of unlabelled R1881 and the positive control compounds testosterone and dihydrotestosterone were also assessed and IC_{50} values calculated where possible. No competition for the binding of R1881 at the androgen receptor was seen for iprodione or its metabolites RP32490, RP36112, RP36114, RP36115, RP36118 and RP36119. Some competition was seen with the iprodione metabolite RP25040 at the two highest concentrations investigated. IC_{50} values were determined for R1881 (1.7 nM), testosterone (694 nM), dihydrotestosterone (187 nM) and the metabolite RP25040 (115800 nM). The study therefore indicates that iprodione and its metabolites RP32490, RP36112, RP36114, RP36115, RP36118 and RP36119 do not interact the human androgen receptor. The study indicates a very weak interaction with the androgen receptor by the iprodione metabolite RP25040 only at very high concentrations; this is considered unlikely to be of biological significance.

I. MATERIAL AND METHODS

A. MATERIALS

1 Test Material:

Lot/Batch no.: iprodione (batch TV3015C)
iprodione metabolite RP25040 (batch TV2840/F)
iprodione metabolite RP32490 (batch GD8309)
iprodione metabolite RP36112 (batch BES1526)
iprodione metabolite RP36114 (batch BES308)
iprodione metabolite RP36115 (batch BES129)
iprodione metabolite RP36118 (batch GD6842)
iprodione metabolite RP36119 (batch GD8002)

Purity: 98.1% [iprodione]

CAS: 36734-19-7

Stability: Verified by Sponsor

2 Vehicle: DMSO

3 Reference compounds: [^{3}H]-R1881 (methyltrienolone / Metribolone)
Positive control: Testosterone
Positive control: Dihydrotestosterone

4 Test system Androgen receptors isolated from the T47D Human ductal breast carcinoma cell line

B. STUDY DESIGN AND METHODS

1 Study dates

Study Initiation Date: 19th April 1999 (start of the experimental phase)
Study Completion Date: 26th May 1999 (end of the experimental phase)

2 Assay performance

Androgen receptors were isolated from the T47D human ductal breast carcinoma cell line, cultured in one hundred 17 cm^2 cell culture flasks. The protein content was determined and was standardised at a concentration of 300 µg/tube for the binding assay. The competition assays were performed using a constant amount (2 nM) of tritiated R1881 (methyltrienolone / Metribolone) and a series of concentrations of the test substances (iprodione and its seven metabolites RP25040, RP32490, RP36112, RP36114, RP36115, RP36118 and RP36119) and the positive control substances (testosterone and dihydrotestosterone). Concentrations of between 10^{-11} and 10^{-5} M were assessed for iprodione, RP32490, RP36115 and dihydrotestosterone; concentrations of between 10^{-11} and 10^{-4} M were assessed for RP25040, RP36112, RP36114, RP36118, RP36119 and testosterone. Free and androgen receptor-bound ^{3}H-R1881 were separated following precipitation of the free fraction with activated charcoal. Levels of receptor-bound ^{3}H-R1881 were quantified by liquid scintillation counting.

3 Statistics

Data were fitted according to a one-site competition model using GraphPad Prism version 2.0. Where applicable, IC50 values were calculated directly from the resulting graphs for R1881, testosterone and dihydrotestosterone. Competition was incomplete within the concentration range for RP25040 and an IC50 value could not be calculated. For RP25040 it was assumed that 10% of total binding represented complete inhibition in order to obtain an IC50 value.

II. RESULTS AND DISCUSSION

A STABILITY TESTING

No differences were observed between UV spectra at the two time points, indicating that the test substances were stable over the course of the assay.

B RECEPTOR BINDING ASSAY

IC_{50} values were determined for unlabelled R1881 and for the positive control substances (testosterone and dihydrotestosterone). Competition was incomplete within the concentration range for RP25040 and an IC_{50} value could not be calculated; some displacement of ^{3}H-R1881 by RP25040 was seen only at the highest concentrations of 10^{-4} and 10^{-5} M. For RP25040 it was assumed that 10% of total binding represented complete inhibition in order to obtain an IC_{50} value. iprodione and the metabolites RP32490, RP36112, RP36114, RP36115, RP36118 and RP36119 did not show any evidence of competition with ^{3}H-R1881 for the androgen receptor under the conditions of this assay. IC50 values for unlabelled R1881 and the positive control substances (testosterone and dihydrotestosterone) confirmed the sensitivity of the assay. IC_{50} values are shown in the table below.

Table 5.8.2-4: Androgen receptor binding assay results

Substance		**IC50 value** (nM)
Reference substance	Unlabelled R1881	1.7
Positive control	Testosterone	694
	Dihydrotestosterone	187
iprodione		-[b]
iprodione metabolite	RP25040	115800[a]
	RP32490	-[b]
	RP36112	-[b]
	RP36114	-[b]
	RP36115	-[b]
	RP36118	-[b]
	RP36119	-[b]

[a] *competition was incomplete within the concentration range*
[b] *no interaction seen within the concentration range tested*

E DISCUSSION

No evidence for the interaction of iprodione or its metabolites RP32490, RP36112, RP36114, RP36115, RP36118 and RP36119 with the human androgen receptor were seen under the conditions of this competitive binding assay performed *in vitro* in the T47D human cell line and tritiated R1881 (methyltrienolone / Metribolone). A very weak interaction with the androgen receptor was seen for the iprodione metabolite RP25040; however this was only seen at the highest concentrations investigated (10^{-4} and 10^{-5} M). IC50 values for unlabelled reference compound and the positive control substances (testosterone and dihydrotestosterone) confirmed the sensitivity of the assay. The IC50 value calculated for metabolite RP25040, which is several orders of magnitude greater than the positive controls, is therefore considered unlikely to be of any biological significance.

III. CONCLUSION

This study therefore indicates that iprodione and its metabolites RP32490, RP36112, RP36114, RP36115, RP36118 and RP36119 do not interact the human androgen receptor. The study indicates a very weak interaction with the androgen receptor by the iprodione metabolite RP25040.

Report: CA 5.8.2/4
[redacted]., 1998b
Iprodione - Hormonal measurements in adult male Sprague Dawley rats following administration of Iprodione by gavage
B003656
Guidelines: none
GLP: yes

Executive Summary

The effect of iprodione administration on circulating testosterone and luteinising hormone (LH) levels was assessed in a study in adult male Sprague-Dawley rats. Groups of 75 rats were gavaged with a single dose of iprodione (in 0.4% methylcellulose) at dose levels of 0 (vehicle control), 70 or 300 mg/kg bw. An additional group of rats was gavaged with the positive control substance ketoconazole (300 mg/kg bw). Blood samples (15 rats/group/sample time) were taken at 2, 4, 6, 10 and 24 hours following administration and assessed for plasma levels of testosterone and luteinising hormone using radioimmunoassay. Plasma levels of testosterone were significantly reduced at dose levels of 70 and 300 mg/kg bw iprodione as early as two hours post dosing. At both iprodione dose levels, plasma testosterone levels returned to normal within six hours. Administration of ketoconazole resulted in a marked and statistically significant reduction in plasma testosterone at four hours after dosing and a significant increase in plasma LH concentration at 24 hours after dosing. The author concludes that the increase in circulating LH levels resulting from the repeated administration of iprodione is a causative factor in the induction of Leydig cell tumours seen in the rat carcinogenicity study.

I. MATERIAL AND METHODS

A. MATERIALS

1 Test Material:

Description:	iprodione, white granules
Lot/Batch no.:	9426801
Purity:	96.5 g/kg
CAS:	36734-19-7
Stability:	Stability verified over the study period

2 Dosing:

Dose levels:	0, 70, 300 mg/kg bw [iprodione]
Dosing vehicle:	0.4% aqueous methylcellulose
Dose volume:	5 mL/kg bw
Dosing period:	1 day
Positive control:	Ketoconazole

3 Test animals

Species:	Rat
Strain:	Crl:CD(SD)BR
Sex:	Male (10/group)
Age:	13 weeks at the time of dosing
Weight:	362-476 g at the time of dosing
Source:	Charles River, France
Acclimation period:	8 days
Diet:	Certified Rodent Pellet diet M20 contrôlé; *ad libitum*
Water:	Filtered and softened municipal tap water; *ad libitum*
Housing:	Individual

4 Environmental conditions:

Cage:	Steel wire mesh, suspended
Temperature:	20-24°C
Humidity:	40-70%
Photoperiod:	12/12 hours light/dark cycle
Ventilation:	10-15 air changes / hour

B. STUDY DESIGN AND METHODS

1 Study dates

Study Initiation Date: 1st July 1998
Study Completion Date: 24th July 1998

3 Assay performance

Groups of ten adult male rats were gavaged with a single dose of iprodione (in 0.4% aqueous methylcellulose) at dose levels of 0 (vehicle control), 70 or 300 mg/kg bw; additional groups were gavaged with a single dose of the positive control substance ketoconazole (300 mg/kg bw). Dosing was randomised and performed at 3-minute intervals to standardise the time between dosing at blood sampling. Blood samples were taken from ten rats/group at sample times of 2, 4, 10 and 24 hours following administration of the vehicle control or iprodione; and at 4 and 24 hours following administration of ketoconazole. Animals were observed daily for mortality, moribundity and clinical signs. Bodyweights were recorded. Blood samples taken from the retro-orbital plexus were analysed for testosterone and LH by radioimmunoassay. The testes (paired) from each animal were weighed following termination.

4 Statistics

The results of hormone analysis were intercompared using Bartlett's test for homogeneity of variances, analysis of variance (ANOVA). If Bartlett's test indicated homogenous variances and the ANOVA was significant, group and control means were intercompared using Dunnett's test. If Bartlett's test indicated homogenous variances and the ANOVA was significant, the group and control means were intercompared using Dunnett's test. If Bartlett's test indicated homogenous variances and the ANOVA was significant, a log transformation was performed. When Bartlett's test was significant following transformation, non-parametric statistical analyses (Kruskal-Wallis, Mann-Whitney tests) were performed.

II. RESULTS AND DISCUSSION

A DOSE FORMULATION ANALYSIS

Actual concentrations, homogeneity and stability of dose formulations were within acceptable limits.

B IN LIFE OBSERVATIONS

No deaths occurred. No treatment-related signs were observed during the study period in rats administered iprodione. Signs of toxicity (piloerection, hypothermia, facial soiling, lacrimation) were observed in rats of the positive control group.

C HORMONE LEVELS

Treatment with iprodione at 70 and 300 mg/kg bw resulted in a significant and dose-dependent reduction of plasma testosterone concentration from two hours after dosing and persisting to four hours after dosing at the higher dose level. Treatment with iprodione also resulted in a significant and dose-dependent increase in plasma LH concentration at two and four hours after dosing at 300 mg/kg bw and at four hours after dosing at the higher dose level. No significant effects on plasma testosterone or LH concentration were seen in either iprodione-treated group at 6-24 hours after dosing. Administration of a single gavage dose of ketoconazole (300 mg/kg bw) resulted in a marked and statistically significant reduction in plasma testosterone at four hours after dosing and a significant increase in plasma LH concentration at 24 hours after dosing.

Table 5.8.2-5: Plasma hormone levels

Dose level (mg/kg bw)		**0**	**70**	**300**	**Ketoconazole (300)**
Testosterone (ng/mL)	**2h**	6.38 ± 2.76	**2.63** ± 1.37**	**1.61** ± 0.66**	
	4h	1.96 ± 1.29	1.40 ± 0.66	**0.81* ± 0.37**	**0.34** ± 0.13**
	6h	2.65 ± 2.22	1.70 ± 0.91	1.95 ± 1.92	
	10h	2.51 ± 1.14	3.28 ± 1.69	2.56 ± 0.86	
	24h	2.56 ± 0.94	2.59 ± 1.54	3.79 ± 1.95	1.88 ± 1.02
LH (ng/100 µL)	**2h**	0.21± 0.03	0.26 ± 0.08	**0.28* ± 0.07**	
	4h	0.22 ± 0.04	**0.30* ± 0.07**	**0.34** ± 0.06**	0.22 ± 0.04
	6h	0.25 ± 0.05	0.29 ± 0.08	0.32 ± 0.10	
	10h	0.18 ± 0.06	0.23 ± 0.09	0.24 ± 0.06	
	24h	0.21 ± 0.05	0.22 ± 0.10	0.26 ± 0.06	**0.34** ± 0.09**

**significantly different to controls (p<0.05); **p<0.01*

D DISCUSSION

Administration of a single gavage dose of iprodione to adult male rats at dose levels of 70 and 300 mg/kg bw was found to cause a rapid and transient decrease in plasma testosterone concentration. Effects were apparent within 2-4 hours of dosing; plasma testosterone levels returned to levels comparable to the vehicle controls within four hours (at 70 mg/kg bw) or six hours (at 300 mg/kg bw). At both iprodione dose levels, a transient homeostatic increase in plasma LH was also observed. The effect on plasma LH concentration was apparent from two hours (at 300 mg/kg bw) or at four hours (at 70 mg/kg bw) after dosing and returned to levels comparable to the vehicle controls within six hours at both dose levels. Administration of a single gavage dose of the positive control substance ketoconazole resulted in a more marked and statistically significant reduction in plasma testosterone at four hours after dosing and a significant increase in plasma LH concentration at 24 hours after dosing.

III. CONCLUSION

Administration of a single dose of iprodione to the adult male rat results in a transient reduction in circulating testosterone levels and a consequent homeostatic increase in the levels of circulating LH. The author concludes that the increase in circulating LH levels resulting from the repeated administration of iprodione is a causative factor in the induction of Leydig cell tumours seen in the rat carcinogenicity study.

Report: CA 5.8.2/5
[redacted]., 2001b
Iprodione - Hormonal measurements in adult male Sprague Dawley rats following a single administration of Iprodione by gavage
C019327
Guidelines: none
GLP: yes

Executive Summary

The effect of iprodione administration on circulating testosterone and luteinising hormone (LH) levels was assessed in a study in adult male Sprague-Dawley rats. Groups of 75 rats were gavaged with a single dose of iprodione (in 0.4% methylcellulose) at dose levels of 0 (vehicle control), 70 or 300 mg/kg bw. Blood samples (15 rats/group/sample time) were taken at 1, 2, 4 and 24 hours following administration and assessed for plasma levels of testosterone and luteinising hormone using radioimmunoassay. Plasma levels of testosterone were significantly reduced at dose levels of 70 and 300 mg/kg bw iprodione as early as 30 minutes post dosing. At both iprodione dose levels, plasma testosterone levels returned to normal within 24 hours. The author concludes that the increase in circulating LH levels resulting from the repeated administration of iprodione is a causative factor in the induction of Leydig cell tumours seen in the rat carcinogenicity study.

I. MATERIAL AND METHODS

A. MATERIALS

1 Test Material:

Description:	iprodione, white granules
Lot/Batch no.:	0014801
Purity:	97.4%
CAS:	36734-19-7
Stability:	Stability verified for 28 days

2 Dosing:

Dose levels:	0, 70, 300 mg/kg bw
Dosing vehicle:	0.4% aqueous methylcellulose
Dose volume:	5 mL/kg bw
Dosing period:	1 day

3 Test animals

Species:	Rat
Strain:	Crl:CD(SD)BR
Sex:	Male (75/group)
Age:	Approximately 13 weeks at the time of dosing
Source:	IFFA-Credo, France
Acclimation period:	14 days
Diet:	Ground and irradiated UAR Certified Rodent Meal; *ad libitum*
Water:	Filtered and softened municipal tap water; *ad libitum*
Housing:	Individual

4 Environmental conditions:

Cage:	Steel wire mesh, suspended
Temperature:	20-24°C
Humidity:	40-70%
Photoperiod:	12/12 hours light/dark cycle
Ventilation:	10-15 air changes / hour

B. STUDY DESIGN AND METHODS

1 Study dates

Study Initiation Date: 9th February 2001
Study Completion Date: 27th April 2002

2 Assay performance

Animals were habituated to the dosing procedure received by the administration of daily gavage doses of water for seven days prior to the administration of a single gavage dose of iprodione (in 0.4% methylcellulose) to groups of 60 adult male rats at dose levels of 0 (vehicle control), 70 or 300 mg/kg bw. Dosing was randomised and performed at 2-minute intervals to standardise the time between dosing at blood sampling. Blood samples were taken from 15 rats/group at sample times of 1, 2, 4 and 24 hours following administration. Animals were observed daily for mortality, moribundity and clinical signs. Bodyweights were recorded. Blood samples taken from the retro-orbital plexus were analysed for testosterone and LH by radioimmunoassay.

3 Statistics

For testosterone and LH levels, group variances were compared using Bartlett's test. When Bartlett's test was not significant, ANOVA was performed. When the variance was not significant, the means of the control and treated groups were homogenous. When the variance was significant, means of the control and treated groups were compared using Dunnett's test. When Bartlett's test was significant, data were log-transformed and compared using Bartlett's test. When the variance of the transformed data was not significant, the means of the control and treated groups were homogenous. When the variance was significant, means of the control and treated groups were compared using Dunnett's test.

II. RESULTS AND DISCUSSION

A DOSE FORMULATION ANALYSIS

Actual concentrations and homogeneity of dose formulations were within acceptable limits.

B IN LIFE OBSERVATIONS

No treatment-related signs were observed during the study period. One animal died during blood sampling.

C HORMONE LEVELS

In animals dosed with 300 mg/kg bw iprodione, plasma testosterone levels were significantly reduced at 0.5, 1, 2 and 4 hours. In animals dosed with 70 mg/kg bw iprodione, plasma testosterone levels were significantly reduced at 0.5, 1 and 2 hours.

Table 5.8.2-6: Plasma hormone levels

Dose level (mg/kg bw)		**0**	**70**	**300**
Testosterone (ng/mL)	**0.5h**	7.1 ± 3.8	**3.7** ± 2.3**	**3.6** ± 1.6**
	1h	5.7 ± 3.8	**2.9** ± 2.0**	**1.9** ± 1.1**
	2h	2.5 ± 0.8	**0.9** ± 0.6**	**1.1** ± 0.7**
	4h	2.6 ± 1.4	1.7 ± 1.2	**1.2** ± 0.7**
	24h	4.7 ± 2.3	4.1 ± 2.3	5.0 ± 2.2
LH (ng/mL)	**0.5h**	2.0 ± 0.6	1.6 ± 0.7	1.8 ± 0.7
	1h	1.4 ± 0.5	1.6 ± 0.6	1.6 ± 0.6
	2h	1.3 ± 0.3	1.5 ± 0.4	**1.8** ± 0.5**
	4h	2.0 ± 0.4	2.2 ± 0.5	**2.6** ± 0.7**
	24h	2.0 ± 0.6	1.9 ± 0.4	2.1 ± 0.5

**significantly different to controls (p<0.05); **p<0.01*

D DISCUSSION

Administration of a single gavage dose of iprodione to adult male rats at dose levels of 70 and 300 mg/kg bw was found to cause a rapid and transient decrease in plasma testosterone concentrations. Effects were apparent within 30 minutes of dosing; plasma testosterone levels returned to levels comparable to the vehicle controls within four hours (70 mg/kg bw) or 24 hours (300 mg/kg bw). At the higher dose level of 300 mg/kg bw, a transient homeostatic increase in plasma LH was also observed. The effect on plasma LH concentration was apparent from two hours after dosing and returned to levels comparable to the vehicle controls within 24 hours.

III. CONCLUSION

Administration of a single dose of iprodione to the adult male rat results in a transient reduction in circulating testosterone levels and a consequent homeostatic increase in the levels of circulating LH. The author concludes that the increase in circulating LH levels resulting from the repeated administration of iprodione is a causative factor in the induction of Leydig cell tumours seen in the rat carcinogenicity study.

Report: CA 5.8.2/6
[redacted]., 2002c
Iprodione - Hormonal measurements following a 14-day treatment period with Iprodione by gavage in the rat
C024675
Guidelines: none
GLP: yes
(certified by Groupe Interministeriel des Produits Chimiques, France)

Executive Summary

The effect of iprodione administration on circulating testosterone and luteinising hormone (LH) levels was assessed in a study in adult male Sprague-Dawley rats. Groups of 60 rats were gavaged with iprodione (in 0.4% methylcellulose) on 14 consecutive days at dose levels of 0 (vehicle control), 6, 70 or 300 mg/kg bw/d. Blood samples (15 rats/group/sample time) were taken at 1, 2, 4 and 24 hours following administration of the final iprodione dose and assessed for plasma levels of testosterone and luteinising hormone using radioimmunoassay. Plasma levels of testosterone were significantly reduced at a dose level of 300 mg/kg bw/d (at 1, 2 and 4 hours post-dosing) and at a dose level of 70 mg/kg bw/d (at 2 hours post dosing only). At both iprodione dose levels, plasma testosterone levels returned to normal within 24 hours. Plasma testosterone levels at a dose level of 6 mg/kg bw/d were unaffected by treatment. Plasma LH levels were significantly elevated on Day 14 at 70 and 300 mg/kg bw/d (at 4 hours post-dosing only); at both dose levels, plasma testosterone levels returned to normal within 24 hours. Plasma LH levels at a dose level of 6 mg/kg bw/d were unaffected by treatment. The administration of iprodione in the rat therefore results in a transient reduction in circulating testosterone levels and a consequent homeostatic increase in the levels of circulating LH. The author concludes that the increase in circulating LH levels resulting from the repeated administration of iprodione is a causative factor in the induction of Leydig cell tumours seen in the rat carcinogenicity study.

I. MATERIAL AND METHODS

A. MATERIALS

1 Test Material:

Description:	iprodione, white granules
Lot/Batch no.:	0014801
Purity:	97.4%
CAS:	36734-19-7
Stability:	Stability verified for 28 days

2 Dosing:

Dose levels:	0, 6, 70, 300 mg/kg bw/d
Dosing vehicle:	0.4% aqueous methylcellulose
Dose volume:	5 mL/kg bw
Dosing period:	14 days

3 Test animals

Species:	Rat
Strain:	Crl:CD(SD)BR
Sex:	Male (60/group)
Age:	Approximately 13 weeks at the time of first dosing
Source:	IFFA-Credo, France
Acclimation period:	14 days
Diet:	Ground and irradiated UAR Certified Rodent Meal; *ad libitum*
Water:	Filtered and softened municipal tap water; *ad libitum*
Housing:	Individual

4 Environmental conditions:

Cage:	Steel wire mesh, suspended
Temperature:	20-24°C
Humidity:	40-70%
Photoperiod:	12/12 hours light/dark cycle
Ventilation:	15 air changes / hour

B. STUDY DESIGN AND METHODS

1 Study dates

Study Initiation Date: 10th October 2001
Study Completion Date: 13th February 2002

2 Assay performance

Groups of 60 adult male rats were gavaged with iprodione (in 0.4% aqueous methylcellulose) at dose levels of 0 (vehicle control), 6, 70 or 300 mg/kg bw/d on 90 consecutive days. On the final day of dosing, dosing was randomised and performed at 2-minute intervals to standardise the time between dosing at blood sampling. Blood samples were taken from 15 rats/groups at sample times of 1, 2, 4 and 24 hours following administration of the final dose. Animals were observed daily for mortality, moribundity and clinical signs. Bodyweights and food consumption were recorded weekly. Blood samples were analysed for testosterone and LH by radioimmunoassay.

3 Statistics

Statistical analyses were performed at RTI International, NC, USA. Bodyweight and food consumption data were compared using either parametric ANOVA and Dunnett's test or by non-parametric values (Kruskal-Wallis and Mann-Whitney U test). The homogeneity of variance was examined using Bartlett's test. If Bartlett's test indicated lack of homogeneity of variance, non-parametric methods were used. The Kruskal-Wallis test was used to test overall treatment group differences, followed by individual Mann-Whitney U-tests for exposed vs. control group comparisons when the overall treatment effect was significant ($p<0.05$). If Bartlett's test did not reject the hypothesis of homogenous variance, standard ANOVA techniques were used. For hormone measurements, treatment groups were compared using either parametric ANOVA and Dunnett's test or by non-parametric values (Kruskal-Wallis and Mann-Whitney U test).

II. RESULTS AND DISCUSSION

A DOSE FORMULATION ANALYSIS

Actual concentrations and homogeneity of dose formulations were within acceptable limits.

B IN LIFE OBSERVATIONS

No treatment-related mortality occurred during the study period. A small number of deaths occurred and were attributed to dosing error. Signs of toxicity were limited to soiled perineal fur in eight rats at the highest dose level of 300 mg/kg bw/d. Mean bodyweight gain was significantly reduced at 300 mg/kg bw/d; mean food consumption was also reduced in this group. No effects on bodyweight gain or food consumption were observed at dose levels of 6 or 70 mg/kg bw/d.

C TERMINAL FINDINGS

In animals dosed with 300 mg/kg bw/d iprodione, plasma testosterone levels were significantly reduced at 1, 2 and 4 hours post dosing. In animals dosed with 70 mg/kg bw/d iprodione, plasma testosterone levels were significantly reduced only at 2 hours post dosing. In both groups, plasma testosterone levels were comparable to the vehicle control group at 24 hours. In animals dosed with 70 and 300 mg/kg bw/d iprodione, plasma LH levels were significantly reduced at 4 hours post dosing. In both groups, plasma LH levels were comparable to the vehicle control group at 24 hours. A significant reduction in LH level at 6 mg/kg bw/d at 24 hours post dosing is not considered to be related to treatment with iprodione.

Table 5.8.2-7: Weight gain and plasma hormone levels

Dose level (mg/kg bw/d)		**0**	**6**	**70**	**300**
Weight gain (g)	**d1-7**	14.8 ± 16.2	16.3 ± 10.7	16.0 ± 10.0	11.9 ± 23.9
	d7-13	18.0 ± 9.9	19.8 ± 7.7	16.7 ± 6.9	**2.4** ± 14.1**
	d1-14	32.8 ± 25.1	36.1 ± 16.3	32.7 ± 14.1	14.3 ± 28.5
Testosterone (ng/mL)	**1h**	3.46 ± 1.29	2.86 ± 1.27	2.57 ± 1.14	**2.20* ± 0.93**
	2h	3.82 ± 1.69	3.47 ± 1.60	**2.14* ± 0.69**	**2.39* ± 1.29**
	4h	2.23 ± 1.23	2.21 ± 1.38	2.56 ± 1.13	**1.15** ± 0.51**
	24h	3.26 ± 1.48	3.14 ± 2.17	3.19 ± 2.15	4.42 ± 3.74
LH (ng/100 µL)	**1h**	1.07 ± 0.40	1.34 ± 0.47	1.13 ± 0.46	1.30 ± 0.28
	2h	0.99 ± 0.33	1.08 ± 0.41	1.31 ± 0.52	1.38 ± 0.72
	4h	1.82 ± 0.61	1.76 ± 0.30	**2.26** ± 0.40**	**2.80** ± 0.99**
	24h	1.65 ± 0.28	**1.22 ** ± 0.34**	1.57 ± 0.47	1.89 ± 0.77

**significantly different to controls ($p<0.05$); **$p<0.01$*

D DISCUSSION

Administration of iprodione to adult male rats at dose levels of 70 and 300 mg/kg bw/d on fourteen consecutive days was found to cause a transient decrease in plasma testosterone concentration and a transient, homeostatic increase in plasma LH. Effects on plasma hormone concentrations were reversible within 24 hours of dosing. Effects at 300 mg/kg bw/d were associated with signs of general toxicity and reduced weight gain. No effects on plasma testosterone or LH levels were seen at the low dose level of 6 mg/kg bw/d iprodione.

III. CONCLUSION

Administration of iprodione in the adult male rat results in a transient reduction in circulating testosterone levels and a consequent homeostatic increase in the levels of circulating LH. The author concludes that the increase in circulating LH levels resulting from the repeated administration of iprodione is a causative factor in the induction of Leydig cell tumours seen in the rat carcinogenicity study.

Report: CA 5.8.2/7
[redacted]., 2002d
Iprodione - Measurement of leydig cell proliferation following a 14-day treatment period with Iprodione by gavage in the rat
C025273
Guidelines: none
GLP: yes
(certified by Groupe Interministeriel des Produits Chimiques, France)

Executive Summary

The effect of iprodione administration on testicular interstitial (Leydig) cell proliferation was investigated in this study performed in adult male Sprague-Dawley rats. Groups of fifteen rats were administered iprodione (in 0.4% aqueous methylcellulose) by gavage on fourteen consecutive days at dose levels of 0 (vehicle control), 6, 70 or 300 mg/kg bw/d. A positive control group was similarly administered the androgen receptor antagonist flutamide at a dose level of 150 mg/kg bw/d. Animals were sacrificed at 24 hours following administration of the final dose. One week before sacrifice, animals were implanted subcutaneously with osmotic mini-pumps containing bromodeoxyuridine (BrdU). At termination, gross necropsy was performed on all animals; the weights of the brain and testes were recorded. Leydig cell proliferation was assessed by visual measurement of incorporation of BrdU following immunohistochemical staining. Administration of iprodione at a dose level of 300 mg/kg bw/d resulted in signs of toxicity (anogenital soiling) and reduced bodyweight gain over the study period; reduced weight gain over the second half of the study period was also observed at an iprodione dose level of 70 mg/kg bw/d. Mean absolute and relative testes weights were unaffected by treatment with iprodione at all dose levels; significantly reduced testes weights were observed in the positive control group. Assessment of Leydig cell proliferation revealed a statistically significant and dose-related increase in the mean BrdU labelling index in rats treated with 70 and 300 mg/kg bw/d iprodione; a large and statistically significant increase in BrdU labelling was seen in rats administered flutamide. No increase in BrdU labelling was seen in rats administered iprodione at the low dose level of 6 mg/kg bw/d. The results of this study therefore show that the administration of iprodione at dose levels of 70 and 300 mg/kg bw/d for 14 consecutive days caused signs of general toxicity and a significant increase in Leydig cell proliferation. A clear NOAEL of 6 mg/kg bw/d iprodione was therefore determined for this study.

I. MATERIAL AND METHODS

A. MATERIALS

1 Test Material:

Description:	iprodione, white granules
Lot/Batch no.:	0014801
Purity:	97.4%
CAS:	36734-19-7
Stability:	Stability verified for 28 days

2 Dosing:

Dose levels:	0, 6, 70, 300 mg/kg bw/d iprodione 150 mg/kg bw/d flutamide [positive control]
Dosing vehicle:	0.4% aqueous methylcellulose
Dose volume:	5 mL/kg bw
Dosing period:	14 days

3 Test animals

Species:	Rat
Strain:	Crl:CD(SD)BR
Sex:	Male (60/group)
Age:	Approximately 13 weeks at the time of first dosing
Source:	IFFA-Credo, France
Acclimation period:	20 days
Diet:	Ground and irradiated UAR A0C-10 Certified Rodent Meal; *ad libitum*
Water:	Filtered and softened municipal tap water; *ad libitum*
Housing:	Individual

4 Environmental conditions:

Cage:	Steel wire mesh, suspended
Temperature:	20-24°C
Humidity:	40-70%
Photoperiod:	12/12 hours light/dark cycle
Ventilation:	15 air changes / hour

B. STUDY DESIGN AND METHODS

1 Study dates

Study Initiation Date: 7th November 2001
Study Completion Date: 16th May 2002

2 Assay performance

Groups of fifteen adult male Sprague-Dawley rats were gavaged with iprodione (in 0.4% aqueous methylcellulose) at dose levels of 0 (vehicle control), 6, 70 or 300 mg/kg bw/d on fourteen consecutive days. A positive control group was similarly administered the androgen receptor antagonist flutamide at a dose level of 150 mg/kg bw/d. Rats were observed daily for mortality and signs of toxicity; bodyweights and food consumption were measured weekly. One week prior to the scheduled sacrifice, all rats were implanted subcutaneously with an osmotic mini-pump containing bromodeoxyuridine (BrdU). Animals were sacrificed 24 hours following administration of the final dose. Gross necropsy was performed on all animals; weights of the brain and testes (paired) were recorded. The testes and duodenum were investigated microscopically following haematoxylin and eosin staining. Leydig cell and duodenal proliferation were assessed immunohistochemically using anti-BrdU monoclonal antibodies, amplification with a secondary biotinylated antibody, a streptavidin-biotin peroxidase complex and detection using the chromogen diaminobenzene. The duodenum was also investigated as a tissue with a high background proliferation rate. Leydig cell counting on slides prepared from each testes (at least 500 cells per slide; 1000 cells per animal). Counting was performed visually and expressed as the Labelling Index (% of BrdU positive Leydig cells per animal).

3 Statistics

Mean values and associated standard deviations were calculated using SAS v. 8.02). Bartlett's test was used to compare the homogeneity of variances. If Bartlett's test was not significant, means of the control and treated groups were compared using Dunnett's test. If Bartlett's test was significant, means were compared using the Kruskal-Wallis test. If the Kruskal-Wallis test was significant, the Mann-Whitney test was used to compare control and treated groups. For the positive control group, the F test was used to compare homogeneity of variance; means were subsequently compared using the t-test (if the F test was not significant) or the modified t-test (if the F test was significant). For Leydig cell counts, data from the treated group were log transformed if Bartlett's test was significant. If Bartlett's test on the transformed data was significant, the Mann-Whitney test was used; if Bartlett's test on the transformed data was not significant Dunnett's test was used.

II. RESULTS AND DISCUSSION

A DOSE FORMULATION ANALYSIS

Actual concentrations and homogeneity of dose formulations were within acceptable limits.

B IN LIFE OBSERVATIONS

No deaths occurred during the study period. Signs of toxicity (soiled perineal fur) were observed in three rats at the highest dose level of 300 mg/kg bw/d iprodione. Signs of toxicity (including coloured urine, nasal discharge and soiling, salivation, noisy breathing and/or reduced activity) were observed in all animals in the positive control group. Mean bodyweight gain over the study period was significantly reduced in animals administered 300 mg/kg bw/d iprodione; mean bodyweight gain was also reduced in animals administered 70 mg/kg bw/d iprodione over the second half of the study period. In positive control animals, mean weight loss was observed during the first week of the study and reduced weight gain was observed during the second week of the study. Reduced food consumption was also observed in the positive control group. Weight gain and food consumption by rats administered 6 mg/kg bw/d iprodione were unaffected by treatment.

C TERMINAL FINDINGS

Terminal bodyweights, absolute and relative testes weights in iprodione-treated animals were unaffected by treatment. A significantly higher mean testes weight (relative to bodyweight) was seen in rats administered 300 mg/kg bw/d iprodione; however no dose-response relationship is apparent for this finding, the value at 300 mg/kg bw/d is lower than that for rats at 70 mg/kg bw/d and no significant effect is seen for testes weight relative to brain weight in any iprodione-treated group. This finding is therefore not considered to be related to treatment with iprodione. In the flutamide treated group, significantly lower terminal body weight and significantly lower absolute and relative (to brain weight) testes weights were observed. Gross necropsy did not reveal any effects of treatment with iprodione. One rat treated with 300 mg/kg bw/d iprodione had small testes (bilateral) which is considered to be an incidental finding. Small prostate and seminal vesicle were observed in twelve rats in the positive control group. Histopathology of the testes did not reveal any treatment-related findings; diffuse tubular atrophy was noted in the testes of the single rat administered 300 mg/kg bw/d iprodione with gross observation of small testes; this is considered to be an incidental finding. Assessment of Leydig cell proliferation revealed a statistically significant and dose-related increase in the BrdU labelling index in rats treated with iprodione at 70 and 300 mg/kg bw/d; no effect of treatment was seen at a dose level of 6 mg/kg bw/d iprodione. A marked and statistically significant increase in the BrdU labelling index was seen in the positive control group.

Table 5.8.2-8: Summary of findings

Dose level (mg/kg bw/d)	**0**	**6**	**70**	**300**	**Flutamide (150)**
Weight gain (g) Day 7	3.39 ± 1.43	3.37 ± 1.48	3.21 ± 1.78	**0.39** ± 2.92**	**-5.87** ± 2.47**
Weight gain (g) Day 14	3.25 ± 1.19	3.63 ± 1.06	**1.34** ± 4.29*	**1.39** ± 3.55**	0.85 ± 5.28
Terminal bodyweight (g)	478.5 ± 32.38	487.0 ± 30.47	467.6 ± 35.99	452.8 ± 25.61	**417.6** ± 51.59**
Testes weight (g)	3.440 ± 0.2174	3.523 ± 0.2197	3.565 ± 0.4558	3.460 ± 0.6673	**2.990** ± 0.3465**
Testes weight (relative to body weight)	0.721 ± 0.0689	0.725 ± 0.0593	0.783 ± 0.0798	0.767* ± 0.1509	0.721 ± 0.0906
Testes weight (relative to brain weight)	163.633 ± 11.8935	170.907 ± 11.0914	174.785 ± 22.8607	170.213 ± 32.9099	**148.695** ± 15.2187**
Testes small (#)	-	-	-	1	-
Prostate small (#)	-	-	-	-	12
Seminal vesicles small (#)	-	-	-	-	12
Testes atrophy (#)	-	-	-	1	-
Leydig cell BrdU labelling index	5.0 ± 1.16	5.7 ± 1.61	**6.8** ± 2.89*	**8.7** ± 1.61**	**17.0** ± 12.13**

**significantly different to controls ($p<0.05$); **$p<0.01$*

D DISCUSSION

Administration of iprodione by gavage to adult male rats at dose levels of 70 and 300 mg/kg bw/d on fourteen consecutive days was found to cause a dose-related and significant increase in Leydig cell proliferation, as assessed by the BrdU labelling index. Organ weight and histopathology were unaffected by treatment. Findings were associated with general toxicity at 300 mg/kg bw/d (clinical signs, reduced body weight gain) and at 70 mg/kg bw/d (reduced bodyweight gain). No effect on Leydig cell proliferation was seen at a dose level of 6 mg/kg bw/d iprodione. A marked and statistically significant effect on Leydig cell proliferation was seen in rats administered the androgen receptor antagonist flutamide at a dose level of 150 mg/kg bw/d.

III. CONCLUSION

Administration of iprodione to adult male rats at dose levels of 70 and 300 mg/kg bw/d on fourteen consecutive days was found to cause a dose-related and significant increase in Leydig cell proliferation in the absence of other testicular effects. A clear NOAEL of 6 mg/kg bw/d iprodione was determined for this study.

Report: CA 5.8.2/8
[redacted] 1996b
Cell proliferation in rat testes
R014642
Guidelines: none
GLP: no

Executive Summary

This short study performed by Pathology Associates International (PAI) relates to some additional histopathology work using tissues from a toxicity study performed at Research Triangle Institute (RTI) and entitled "Toxicity testing of a fungicide, iprodione, in adult male male CD Sprague Dawley rats" (BASF DocID C024395). In the study performed at RTI, groups of animals were treated by gavage with iprodione (600 mg/kg/day) or flutamide (150 mg/kg/day) for 30 days and were then subject to necropsy the following day. A control group and a group pair-fed to iprodione were also included in the study and both groups received the vehicle alone by gavage for 30 days before necropsy. Histopathological examination of the testes from these control, pair-fed, iprodione or flutamide animals showed interstitial cell hyperplasia in the flutamide treated animals only. In particular, no interstitial cell hyperplasia was detected in the iprodione treated animals. To further investigate this absence of detectable hyperplasia in the interstitial cells of iprodione treated animals, testes slides from the study performed were re-examined using immunohistochemistry for the proliferating cell nuclear antigen (PCNA) in order to quantitate Leydig cell proliferation.

I STUDY DATES AND METHODS

1 Study dates

August 17, 1996.

2 Methods

Cell proliferation was quantitated in rat testes using proliferating cell nuclear antigen (PCNA) immunohistochemistry. The proliferating index (PI) was determined by scoring the number of Leydig cells in G 1 + S + G2 + M phases of the cell cycle and expressing the PI as a percentage of total Leydig cells counted in 50 fields; approximately 800 cells were scored per animal. In addition to the cell proliferation analysis, the degree of cell labeling within the seminiferous tubules was given a subjective grade between 0 (none) and 4 (marked). Also, a subjective ranking of Leydig cell hyperplasia between 0 and 4 was given. The number of fields with >10 labeled Leydig cells was considered to be heavily labeled and scored as a “focus” of extensive proliferative activity.

3 Statistics

Statistical analysis consisted of comparing control groups to flutamide groups, and pair-fed controls to iprodione groups using the Student's t-test; $P<0.05$ was considered to be significant. Statistical analysis was performed for each replicate experiment, i.e., A and B.

II. RESULTS AND DISCUSSION

The proliferation index did not differ significantly between experiment A and experiment B in either iprodione or pair fed animals. Therefore, the data from the two experiments were pooled for comparison of cell proliferation in iprodione and pair-fed controls. Cell proliferation was significantly increased over controls in the flutamide animals. iprodione induced significant cell proliferation when compared to the pair-fed animals. The degree of labeling within the seminiferous tubules was minimal to mild in the controls, and minimal to moderate in the pair-fed controls, flutamide and iprodione animals. Leydig cell hyperplasia was increased both in incidence and severity in the flutamide animals. Foci of Leydig cell proliferative activity was seen only in flutamide group. Cell proliferation in the iprodione animals was more diffuse than focal.

Flutamide, a positive control for Leydig cell hyperplasia, induced a significant cell proliferative response as evidenced by increased PCNA immunohistochemical labeling as well as hyperplasia as determined histopathologically. Although no noticeable hyperplasia was observed histopathologically following iprodione treatment, a significant increase in PCNA labeling was detected when compared to pair-fed control values. Thus, the PCNA results for iprodione demonstrate a significant increase in Leydig cell proliferation in the absence of detectable hyperplasia at 600 mg/kg/day after oral treatment by gavage.

III. CONCLUSION

Administration of iprodione to adult male rats at dose levels of 600 mg/kg bw/d on thirty consecutive days was found to cause a significant increase in Leydig cell proliferation as determined by PCNA labeling.

Report: CA 5.8.2/9
[redacted], 2012a
Pubertal development and thyroid function in intact juvenile/peripubertal female rats for Iprodione
2012/1364405
Guidelines: EPA 890.1450
GLP: yes
(certified by United States Environmental Protection Agency)
[see 2014/1049126 Shearer K.S. 2014 c]

Executive Summary

The effects of iprodione on mammalian pubertal development were assessed in intact female rats. Groups of 16 juvenile female rats were gavaged with iprodione (in 0.4% aqueous methylcellulose) at dose levels of 0, 150 or 300 mg/kg bw/d for 21 or 22 days from PND 22 to PND 41/42. The dose levels used in the pubertal assay were selected on the basis of a screening assay in which signs of toxicity and mild bodyweight effects were seen at a dose level of 300 mg/kg bw/d. No deaths occurred and no signs of toxicity were observed in the pubertal assay. Mild bodyweight effects were apparent at the highest dose level. Delayed puberty (as assessed by vaginal opening) and delayed oestrus were apparent at the highest dose level. Absolute and relative liver weights were increased at 300 mg/kg bw/d; relative liver weight was increased at 150 mg/kg bw/d. Serum T_4 concentrations were reduced in both treated groups; however other measures of thyroid function including tissue morphology and serum TSH levels were unaffected by treatment.

I. MATERIAL AND METHODS

A. MATERIALS

1 Test Material:

Description:	iprodione technical
Lot/Batch no.:	AE F062470-01-08/ SIN0000217
Purity:	98.1%
CAS:	36734-19-7
Stability:	Stability in dosing vehicle verified for 28 days

2 Dosing:

Dose levels:	0, 150, 300 mg/kg bw/d iprodione
Dose vehicle:	0.4% aqueous methylcellulose
Dose volume:	5 mL/kg bw
Dosing period:	Day 21 PND to Day 41 or 42 PND (21 or 22 doses)

3 Test animals

Species:	Rat
Strain:	Crl:CD(SD)IGS
Sex:	Female (16/group)
Age:	PND 22 (at first dose)
Weight range at dosing:	50.7-71.9 g
Source:	Charles River, NC, USA
Acclimation period:	PND 1-21
Diet:	Teklad Global 16% Protein Rodent Diet, *ad libitum*
Water:	Reverse osmosis purified municipal tap water; *ad libitum*
Housing:	2/cage

4 Environmental conditions:

Cage:	Polycarbonate with microisolator lid
Temperature:	17-24°C
Humidity:	27-64%
Photoperiod:	10/14 hours light/dark cycle

B. STUDY DESIGN AND METHODS

1 Study dates

Study Initiation Date: 19th August 2011
Study Completion Date: 20th December 2011

2 Assay performance

Groups of 16 intact juvenile female rats were gavaged with iprodione (in 0.4% aqueous methylcellulose) at dose levels of 0 (vehicle control), 150 or 300 mg/kg bw/d from PND 22 to PND 42 or 43 (21 or 22 daily doses). Animals were observed daily for mortality, moribundity and clinical signs. Bodyweights were recorded weekly. Vaginal opening was assessed daily from PND 22; oestrus cyclicity was assessed daily (by vaginal smear) from the day at which complete vaginal opening was observed and to termination. Terminal blood samples were taken for the assessment of clinical chemistry parameters, including radioimmunoassay for T_4 and TSH. Gross necropsy was performed on all animals; weights of the adrenals, kidneys, liver, thyroid, ovaries, uterus (wet and blotted) and pituitary were recorded. Histopathology was performed on the ovaries, kidneys, thyroid and uterus. The thyroids were subjectively assessed for follicular height and colloid areas using a five point grading scale. An ovarian follicle count was also performed.

3 Statistics

Descriptive statistics (mean, standard deviation, coefficient of variance, and sample size) were calculated using Microsoft Excel 2003/2007 (Redmond, WA). Datasets were analysed using Statistical Analysis System version 9.2. Studentised residual plots were used to detect possible outliers; Levene's test was used to assess homogeneity of variance. Heterogeneous data were transformed (logarithm, multiplicative inverse, or square root) and if still heterogeneous analyzed using the non-parametric Kruskal-Wallis and Dunn's test. Trend tests were performed on body weight and tissue weight data sets and reported when significant ($p<0.05$) for endpoints that did not show any significant pair wise comparisons. Homogenous datasets (bodyweights, vaginal opening, time of oestrus, cycle length) were analysed using a one-way analysis of variance (ANOVA) followed by pair wise comparisons performed using Dunnett's two tailed t-tests.

Organ weight and clinical chemistry data were analysed using a two-way ANOVA with treatment and necropsy day (if >1 day) as main effects. Pair-wise comparisons were performed using Dunnett's two tailed t-tests. Bodyweight, organ weight and vaginal opening data were analysed using a two-way analysis of covariance (ANCOVA) with PND 21 body weight (allocation body weight) as the co-variable. Pairwise comparisons were performed using Dunnett's two tailed t-tests. Chi-square analyses were used to determine significant differences between the cycling status and percent of animals cycling regularly for treated groups compared to the control group. Statistical analyses of thyroid scoring (colloid area and follicular cell height) were performed by Fisher's Exact test. For all datasets, statistically-significant effects were reported when $p<0.05$.

II. RESULTS AND DISCUSSION

A ASSAY ACCEPTANCE

Data generated from the vehicle control group in this study were within the US EPA performance criteria for age and weight and vaginal opening, organ weight and serum T_4 concentration.

B DOSE FORMULATION ANALYSIS

Actual concentrations and homogeneity of dose formulations were within acceptable limits.

C IN LIFE OBSERVATIONS

No deaths occurred and no signs of toxicity were observed during the study period. A slight reduction in bodyweight was observed in rats at 300 mg/kg bw/d; mean values were 92.6% and 96.3% of controls at Day 14 and at termination, respectively; however values did not attain statistical significance at any time point. A significant delay in vaginal opening was seen at 300 mg/kg bw/d; mean age at initiation and completion of vaginal opening and mean weight at completion of vaginal opening were significantly greater than controls. No effect was apparent at 150 mg/kg bw/d. Mean age at first oestrus was significantly greater in rats at 300 mg/kg bw/d. Significantly lower numbers of rats with regular cycles were observed in both treatment groups; however a dose-response relationship is not apparent for this parameter.

D TERMINAL FINDINGS

Gross necropsy did not reveal any effects of treatment. Relative liver weights were significantly increased at 150 and 300 mg/kg bw/d; absolute liver weight was also increased at 300 mg/kg bw/d. Mean absolute and relative adrenal weights were significantly elevated in both treatment groups. Mean absolute pituitary weight was significantly lower at 300 mg/kg bw/d. Blotted uterine weights were significantly lower in treated groups; however there was no significant difference between wet and blotted organ weights and all weights were within the laboratory's historical control range. Serum T_4 concentrations were significantly reduced in both treated groups; however serum TSH level was unaffected by treatment and no effects were seen on thyroid weight, follicular height or colloid area. No other clinical chemistry findings were affected by treatment. Histopathology revealed a decrease in uterine horn diameter, characterised by a minimal or mild reduction in uterine stromal development in both treated groups. Ovarian follicle counts and thyroid morphological assessment did not reveal any effects of treatment.

Table 5.8.2-9: Female pubertal assay: summary of findings

Dose level (mg/kg bw/d)		**0**	**150**	**300**
Weight gain (g)		108.2 ± 10.1	108.9 ± 11.9	101.8 ± 8.3
Age at vaginal opening initiation (d)		32.8 ± 1.9	34.4 ± 1.9	**36.3* ± 3.0**
Age at vaginal opening completion (d)		32.9 ± 1.8	34.5 ± 1.8	**36.8* ± 3.2**
Weight at vaginal opening completion (g)		119.8 ± 13.6	125.0 ± 11.0	**133.5* ± 16.1**
Age at first oestrus (d)		33.7 ± 2.6	35.1 ± 2.5	**37.2* ± 3.0**
Cycle length (d)		3.7 ± 0.8	4.0 ± 1.1	4.3 ± 1.0
Regularly cycling (%)		100	**69***	**93***
Liver weight (g)		7.43 ± 0.95	8.06 ± 0.84	**8.13* ± 0.70**
Liver weight (%)		4.35 ± 0.24	**4.71* ± 0.31**	**4.97* ± 0.29**
Pituitary weight (mg)		8.9 ± 1.3	8.3 ± 0.9	**7.8* ± 1.2**
Pituitary weight (relative)		5.2 ± 0.6	4.9 ± 0.6	4.8 ± 0.7
Adrenal weight (mg)		43.6 ± 7.6	**53.9* ± 7.5**	**59.4* ± 10.0**
Adrenal weight (relative)		25.5 ± 3.6	**31.6* ± 4.2**	**36.2* ± 4.7**
Uterus weight (mg)	**Wet**	331.4 ± 133.6	281.0 ± 81.7	258.6 ± 140.9
	Blotted	287.2 ± 81.8	255.9 ± 64.7	222.5 ± 96.0
T4 (µg/dL)		3.07 ± 0.74	**0.10* ± 0.06**	**0.20* ± 0.15**
TSH (ng/mL)		1.71 ± 0.76	1.35 ± 0.35	1.49 ± 0.43
Uterus: reduced stromal development (#)		0 [-,-]	2 [2,-]	5 [2,3]

**significantly different to control ($p<0.05$)*
Laboratory grading scale [minimal, mild]

E DISCUSSION

Administration of iprodione to intact juvenile female rats at a dose level of 300 mg/kg bw/d resulted in a mild bodyweight deficit. Absolute and relative liver and adrenal weights were significantly increased in this group compared to vehicle controls, absolute pituitary weight was significantly decreased. Relative liver weight, absolute and relative adrenal weights were significantly increased at 150 mg/kg bw/d. Wet and blotted uterus weights were reduced (but not significantly) in both treated groups. Significant delays in puberty (as assessed by the initiation and completion of vaginal opening) and the time of first oestrus were seen in this group. The proportion of animals with irregular oestrus cycles was significantly increased in both treatment groups, but without a dose-response relationship. Serum T_4 levels were markedly reduced in this group (and in animals administered 150 mg/kg bw/d); however this finding is of unclear toxicological significance as other thyroid parameters were not affected by treatment. Histopathology revealed a reduction in uterine stromal development associated with reduced uterine horn diameter in animals from both treated groups.

III. CONCLUSION

iprodione showed some activity in this assay; initiation and completion of vaginal opening and time to first oestrus were significantly delayed at the highest dose level of 300 mg/kg bw/d.

Report:	CA 5.8.2/10 [redacted]., 2012b The uterotrophic assay (OPPTS 890.1600) for Iprodione 2012/1364406
Guidelines:	EPA 890.1600
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The potential oestrogenic activity of iprodione was assessed in a uterotrophic assay in the immature female rat. Groups of eight juvenile female rats were administered iprodione (in 0.4% aqueous methylcellulose) by gavage at dose levels of 0, 200 or 400 mg/kg bw/d on three consecutive days. An additional group of 16 rats was administered the positive control substance 17α-ethinyl oestradiol by subcutaneous injection. Gross necropsy was performed on all animals; uterine weights (wet and blotted) were recorded at termination. Mean terminal body weight and overall weight gain were significantly reduced in animals administered 400 mg/kg bw/d iprodione; mean weight gain was also significantly reduced at 200 mg/kg bw/d. Mean wet and blotted uterus weights were unaffected by treatment with iprodione in either group; uterus weights in the positive control group were significantly increased compared to the vehicle controls, confirming the sensitivity of the assay. This assay does not therefore provide any evidence for the oestrogenic activity of iprodione.

I. MATERIAL AND METHODS

A. MATERIALS

1 Test Material:

Description:	iprodione technical
Lot/Batch no.:	AE F062470-01-08/ SIN0000217
Purity:	98.1%
CAS:	36734-19-7
Stability:	Stability in dosing vehicle verified for 28 days

2 Dosing:

Dose levels:	0, 200, 400 mg/kg bw/d iprodione
Dosing vehicle:	0.4% aqueous methylcellulose
Dose volume:	5 mL/kg bw
Positive control:	17α-ethinyl oestradiol (vehicle: corn oil) via subcutaneous injection
Dosing period:	Day 21 PND to Day 41 or 42 PND (21 or 22 doses)

3 Test animals

Species:	Rat
Strain:	Crl:CD(SD)IGS
Sex:	Female (8/group)
Age:	PND 19 (at first dose)
Weight range at dosing:	37.9-50.2 g
Source:	Charles River, NC, USA
Acclimation period:	PND 12-18
Diet:	Teklad Global 16% Protein Rodent Diet, *ad libitum*
Water:	Reverse osmosis purified municipal tap water; *ad libitum*
Housing:	2/cage

4 Environmental conditions:

Cage:	Polycarbonate with microisolator lid
Temperature:	21-24°C
Humidity:	45-55%
Photoperiod:	12/12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

1 Study dates

Study Initiation Date: 20th June 2011
Study Completion Date: 5th August 2011

2 Assay performance

Groups of eight juvenile female rats were gavaged with iprodione (in 0.4% aqueous carboxymethylcellulose) at dose levels of 0 (vehicle control), 200 or 400 mg/kg bw/d from PND 19 to PND 21 (three daily doses). The dose levels selected for the pubertal assay were based on the results of a screening study which identified 400 mg/kg bw/d as the MTD. Animals were observed daily for mortality, moribundity and clinical signs. Bodyweights were also recorded daily. Animals were terminated at 24 hours following the final dose; weights of the uterus (wet and blotted) were recorded.

3 Statistics

Descriptive statistics (mean, standard deviation and count) were calculated using MS Excel. Final body weight, body weight gain, and tissue weights were analyzed using SAS (v9.2). Studentised residual plots were used to detect possible outliers and Levene's test was used to assess the homogeneity of variance. Final body weight, body weight gain, and uterine weights were analyzed by one-way ANOVA followed by pair wise comparisons using Dunnett's one-tailed t-test (uterine weights) or Dunnett's two-tailed t-test (final body weight and body weight gain). Statistically significant effects were reported when $p<0.05$. Final body weight, body weight gain, and uterine weights for animals administered the positive control were compared to vehicle control animals by appropriate t-tests. Statistically significant effects were reported when $p<0.05$.

II. RESULT AND DISCUSSION

A ASSAY ACCEPTANCE

The mean blotted uterine weight of animals administered the vehicle (0.4% methylcellulose) was less than 0.09% of body weight, indicating that the study met the performance criterion.

B DOSE FORMULATION ANALYSIS

Actual concentrations and homogeneity of dose formulations were within acceptable limits.

C IN LIFE OBSERVATIONS

No deaths occurred during the study period. Signs of toxicity (loss of coordination, lethargy, unkempt appearance) were observed in rats administered 400 mg/kg bw/d iprodione. No signs of toxicity were observed in rats administered 200 mg/kg bw/d iprodione or in the vehicle or positive control groups. Mean terminal bodyweight in rats administered 400 mg/kg bw/d iprodione was significantly lower than the vehicle controls. Mean weight gains were significantly reduced in animals administered 200 and 400 mg/kg bw/d iprodione.

D TERMINAL FINDINGS

Gross necropsy did not reveal any effects of treatment. Treatment with iprodione had no significant effect on mean wet or blotted uterus weights compared to the vehicle controls. Significant increases in mean wet and blotted uterus weights were seen in the positive control group.

Table 5.8.2-10: Uterotrophic assay: summary of findings

Dose level (mg/kg bw/d)	**0**	**200**	**400**	**Positive control**
Terminal body weight (g)	56.5 ± 5.0	54.2 ± 4.0	**49.8* ± 3.6**	
Weight gain (g)	12.7 ± 1.6	10.0* ± 1.1	**5.9* ± 1.4**	
Wet uterine weight (mg)	28.5 ± 2.9	26.3 ± 2.6	24.7 ± 3.2	**76.9* ± 18.7**
Blotted uterine weight (mg)	26.1 ± 2.7	24.0 ± 3.1	22.1 ± 2.8	**70.3* ± 17.0**

**significantly different to control ($p<0.05$)*

E DISCUSSION

Administration of three consecutive daily gavage doses of iprodione to juvenile female rats at a dose level of 400 mg/kg bw/d resulted in signs of toxicity and a bodyweight deficit; administration of iprodione at a dose level of 200 mg/kg bw/d resulted in a bodyweight deficit. Mean wet and blotted uterus weights were unaffected by treatment in either group. Mean wet and blotted uterus weights in the positive control group (administered 17α-ethinyl oestradiol) were significantly increased compared to the vehicle controls, thereby confirming the sensitivity of the assay.

III. CONCLUSION

No evidence for an oestrogenic effect of iprodione was seen in this uterotrophic assay performed in juvenile female rats.

Report: CA 5.8.2/11
Willoughby J.A., 2013a
Iprodione: Estrogen receptor transcriptional activation (Human Cell Line (HeLa-9903))
2011/1294831
Guidelines: EPA 890.1300
GLP: yes
(certified by United States Environmental Protection Agency)
[see 2014/1049126 Shearer K.S. 2014 c]

Executive Summary

This study assessed the ability of iprodione to act as an agonist of human oestrogen receptor alpha (hERα) using the stably transfected hERα-HeLa-9903 cell line. Concentrations of iprodione tested in this transcriptional activation assay were 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M; concentrations used in the main assay were selected on the basis of a preliminary solubility assay. Due to an accidental spillage in the second assay run, a total of three independent assay runs were performed; data from the second run are not included in subsequent analyses. All assay runs were performed using six replicates per iprodione concentration. Additionally, for each concentration, two replicates incorporated the hERα antagonist ICI 182,780; allowing for the detection of non-specific (non-hERα-mediated) induction of the luciferase gene. The duration of exposure was 24 hours. A complete concentration-response curve for each of four reference compounds (17β-estradiol, 17α-oestradiol, corticosterone and 17α-methyltestosterone) was run each time the transcriptional activation assay was performed. Cytotoxicity (≥20% reduction in cell viability) was observed at the highest concentration of 10^{-4} M iprodione, at a concentration of 10^{-5} M 17α-methyltestosterone and at a concentration of 10^{-4} M corticosterone in the first valid assay run. iprodione did not increase luciferase activity at any of the concentrations investigated in either of the two valid and independent assay runs. It can therefore be concluded that, based on the results of this study, iprodione is not an agonist of human oestrogen receptor alpha (hERα).

I. MATERIAL AND METHODS

A. MATERIALS

1 Test Material:

Description:	iprodione (white powder)
Lot/Batch no.:	AE F062470-01-08 / SIN0000217
Purity:	98.1%
CAS:	36734-19-7
Stability:	Verified by Sponsor

2 Vehicle: DMSO

3 Reference compounds: 17β-oestradiol (strong oestrogen)
17α-oestradiol (weak oestrogen)
Corticosterone (negative control)
17α-methyltestosterone (antagonist)

4 Test system Stably transfected hERα-HeLa-9903 cell line was (Japanese Collection of Research Bioresources (JCRB) Cell Bank. Cells were certified mycoplasma-free.

B. STUDY DESIGN AND METHODS

1 Study dates

Study Initiation Date: 11th July 2011
Study Completion Date: 21st December 2011

2 Assay performance

Stably transfected hERα-HeLa-9903 cells were maintained in Eagle's Minimum Essential Medium (EMEM) without phenol red, supplemented with 60 mg/L Kanamycin and 10% dextran-coated charcoal treated foetal bovine serum (DCC-FBS), in a CO_2 incubator (5% CO_2) at 37±1°C and sub-cultured as required. The cells were suspended in 10% DCC-FBS in EMEM and plated into a 96-well cell culture plate at a density of ≈1 x10^4 and incubated for at least three hours prior to chemical exposure.

After the post-seeding incubation, plates were removed from the incubator and the media aspirated. 75 µL of fresh media and 75 µL of the test chemicals were added to the wells to a final volume of 150 µL/well. All concentrations were tested in replicates of six/plate. In addition, for each concentration, two replicates per plate were prepared that incorporated the hERα antagonist ICI 182,780 to allow for the identification of non-specific luciferase induction. Precipitation of the test materials was assessed visually. Cell viability was monitored by assessment of propidium iodide (PI) uptake in a parallel assay and using 125 µM digitonin as a positive control. Cytotoxic concentrations were excluded from further analyses. Transcriptional activation of the hERα was assessed using a proprietary luciferase assay.

Relative transcriptional activity was assessed by comparing luminescence data to the positive control (1 nM 17β-oestradiol). Data for each concentration were normalized by subtraction of the vehicle control value and compared against the normalized positive control mean value (defined as 100% relative transcriptional activity). For iprodione, the maximum response relative to the positive control (RPCMax) was determined.

3 Statistics

Data are presented as the mean and standard deviation for each valid assay run; the coefficient of variation (CV) was also calculated. Concentration-response data were analysed using Hill's logistic equation, with calculation of the EC50 and maximum induction level using PRISM.

4 Assay acceptance criteria

For each individual run of the transcriptional activation assay, if the calculated RPCmax value was less than 10%, iprodione was considered to have given a negative response for hERα agonism. For each assay run the acceptability of the data was evaluated using the following criteria:

- The mean normalized luciferase signal of the positive control should be at least four times that of the mean vehicle control on each plate.
- The results of the four reference chemicals should be within the acceptable ranges.
- If the acceptability criteria were met, an individual run of the transcriptional activation assay was considered to be definitive

The test substance was considered negative if RPCMax <10% in at least two definitive assay runs. The test substance was considered positive if RPCMax ≥10% in at least two definitive assay.

II. RESULTS AND DISCUSSION

A SOLUBILITY

The highest concentration of iprodione for use in the transcriptional activation assays was identified as 10^{-5} M; higher concentrations exhibited problems with solubility (observed precipitation) and cytotoxicity. Cytotoxicity (≥20% reduction in cell viability) was observed at a concentration of 10^{-4} M iprodione, 10^{-5} M 17α-methyltestosterone and 10^{-4} M corticosterone in the first valid assay run. The final concentrations of iprodione tested in the transcriptional activation assays were 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M, for both valid assay runs

B ASSAY ACCEPTANCE

In both valid assay runs the mean luciferase activity of the positive control substance (1 nM 17β-oestradiol) was greater than four times that of the vehicle control on each plate. In addition, in both assay runs the LogPC50, LogPC10, LogEC50 and Hill slope values for the four reference compounds (17β-oestradiol, 17α-oestradiol, 17α-methyltestosterone and corticosterone) were within the acceptable ranges, with the following minor exceptions:

- In the first assay run, the Hill Slope value for 17α-oestradiol was marginally higher than the specified range (2.5 compared to the specified range of 0.9-2.0)
- In the first assay run, the LogPC10 value for 17α-methyltestosterone was not attained due to cytotoxicity at the highest concentration.
- In the first assay run, the LogPC50 value for 17α-methyltestosterone was not attained.

These deviations from the ranges suggested in the OPPTS guideline were minor and are not considered to have impacted on the interpretation of results. The assay response with 17β-oestradiol, 17α-oestradiol, 17α-methyltestosterone and corticosterone were characteristic of a strong oestrogen, a weak oestrogen, a weak antagonist and a negative compound, respectively. Therefore, both independent runs of the assay were considered to have met the acceptance criteria and are considered to be valid.

C TRANSCRIPTIONAL ACTIVATION ASSAY

In the first assay run, iprodione resulted in a maximal increase in luciferase activity of 3.8 ±1.1; maximal induction was observed at a concentration of 10^{-6} M iprodione, cytotoxicity was observed at 10^{-4} M. In the second valid assay run, iprodione resulted in an increase in luciferase activity of 0.9± 0.4%; maximal induction was seen at an iprodione concentration of 10^{-7} M. Appropriate responses were observed with the reference compounds, confirming the sensitivity of the assay.

Table 5.8.2-11: Oestrogen receptor binding assay results

Material	Concentration (M)	Relative transcriptional activation (% positive control)	
		Run 1	Run 2
iprodione	10^{-11}	-0.5 ±1.1	0.2 ±0.6
	10^{-10}	0.0 ±1.1	0.5 ±0.4
	10^{-9}	1.1 ±1.4	0.8 ±0.8
	10^{-8}	0.4 ±1.4	0.1 ±0.6
	10^{-7}	3.7 ±1.2	0.9 ±0.4
	10^{-6}	3.8 ±1.1	0.7 ±0.6
	10^{-5}	3.1 ±1.6	0.4 ±0.6
	10^{-4}	*	-1.1 ±0.7
17β-oestradiol	10^{-15}	1.2 ±1.5	-0.3 ±0.9
	10^{-14}	1.1 ±1.7	-0.1 ±0.9
	10^{-13}	3.0 ±1.7	1.1 ±1.0
	10^{-12}	1.9 ±2.3	1.2 ±1.1
	10^{-11}	18.6 ±5.1	17.4 ±4.8
	10^{-10}	83.6 ±23.0	95.2 ±27.6
	10^{-9}	128.2 ±35.9	143.8 ±39.3
	10^{-8}	115.3 ±26.9	129.7 ±37.4
17α-oestradiol	10^{-13}	0.1 ±1.4	0.5 ±1.6
	10^{-12}	0.3 ±1.1	0.5 ±0.6
	10^{-11}	1.1 ±1.6	1.5 ±1.3
	10^{-10}	2.4 ±1.7	2.0 ±1.0
	10^{-9}	22.6 ±3.8	28.1 ±5.9
	10^{-8}	107.7 ±23.5	91.3 ±18.7
	10^{-7}	109.2 ±34.8	123.6 ±23.9
	10^{-6}	108.7 ±32.9	97.5 ±20.6
Corticosterone	10^{-11}	0.6 ±1.1	0.2 ±0.5
	10^{-10}	0.1 ±0.8	0.2 ±0.6
	10^{-9}	1.1 ±1.2	0.7 ±1.0
	10^{-8}	-0.2 ±1.0	-0.2 ±0.6
	10^{-7}	3.9 ±1.2	0.5 ±0.4
	10^{-6}	2.8 ±1.5	0.4 ±0.5
	10^{-5}	-0.5 ±1.3	-0.5 ±0.6
	10^{-4}	*	-1.1 ±0.3
17α-methyltestosterone	10^{-12}	-0.5 ±0.8	0.0 ±0.4
	10^{-11}	-0.8 ±0.8	0.5 ±0.4
	10^{-10}	0.4 ±0.6	1.0 ±1.0
	10^{-9}	0.0 ±0.3	0.2 ±0.7
	10^{-8}	4.3 ±3.2	1.9 ±1.3
	10^{-7}	4.4 ±2.1	4.9 ±1.5
	10^{-6}	8.1 ±2.7	11.0 ±3.3
	10^{-5}	*	52.8 ±10.7

**precipitation observed; value not included in subsequent analyses*

E DISCUSSION

The suitable highest concentration of iprodione for use in the transcriptional activation assays was 10^{-5} M, as higher concentrations exhibited problems with solubility (precipitation observed). Cytotoxicity (≥20% reduction in cell viability) was observed at a concentration of 10^{-4} M iprodione in the first assay run. In two valid and independent runs of the transcriptional activation assay, iprodione did not result in an increase in luciferase activity at any of the viable concentrations tested (RPCmax<10%).

III. CONCLUSION

Based on the results of this assay, iprodione is not an agonist of human oestrogen receptor alpha (hERα) in the HeLa-9903 cell system.

Report: CA 5.8.2/12
Willoughby J.A., 2011a
Iprodione: Estrogen receptor binding (rat uterine cytosol)
2011/1294832
Guidelines: EPA 890.1250
GLP: yes
(certified by United States Environmental Protection Agency)
[see 2014/1049126 Shearer K.S. 2014 c]

Executive Summary

The ability of iprodione to interact with the oestrogen receptor (ER) isolated from rat uteri was assessed in this competitive binding assay performed with a single radiolabelled 17β-oestradiol in the presence of increasing concentrations of iprodione and reference substances. Preliminary assessments of precipitation were conducted in order to identify a suitable highest soluble concentration of iprodione for use in the definitive binding assays. Based on the results of the preliminary assay, receptor binding was assessed at iprodione concentrations of 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10-6, 10^{-5} and 10^{-4} M. Binding was assessed in triplicate and in three independent assay runs. Control assays including the radioligand (^{3}H-17β-oestradiol), cytosol and solvent but without the competitor 17β-oestradiol were included in each assay run. The maximal binding of ^{3}H-17β-oestradiol was also assessed. Non-specific binding was assessed in triplicate by determining the extent of radioligand binding in the presence of a 100-fold excess 17β-oestradiol. For each data point, the non-specific binding value was subtracted; values were normalised to total binding (radioactivity eluted from the solvent control tubes) and expressed as percentage specific binding. The duration of incubation at approximately 4°C was 16-20 hours. A complete concentration-response curve was constructed for iprodione, the positive control 17β-oestradiol, the negative control octyltriethoxysilane and the weak positive control 19-norethindrone for each assay run. iprodione was found to be non-interacting in all three valid independent assay runs and is therefore classified as non-interacting with the oestrogen receptor, based on the results of this assay and the guideline criteria.

I. MATERIAL AND METHODS

A. MATERIALS

1 Test Material:

Description:	iprodione (powder)
Lot/Batch no.:	AE F062470-01-08 / SIN0000217
Purity:	98.1%
CAS:	36734-19-7
Stability:	Verified by Sponsor

2 Vehicle: DMSO

3 Reference compounds: [^{3}H]-17β-estradiol specific activity 130.2 Ci/mmol
Positive control: 17-β oestradiol (100% purity)
Negative control: octyltriethoxysilane (99.34% purity)
Weak positive control: 19-norethindrone (99% purity)

4 Test system Rat uterine cytosol oestrogen receptor (sufficient to bind 10-15% of the radioligand)
Radioligand concentration: 1 nM
iprodione concentration: 100 pM-1 mM
Temperature: 4± 2ºC
Incubation time: 16-20 hours
Assay buffer:
Tris 10 mM (pH 7.4)
EDTA 1.5 mM
Glycerol 10%
Protease inhibitor 0.5%
DTT 1 mM

B. STUDY DESIGN AND METHODS

1 Study dates

Study Initiation Date: 11th July 2011
Study Completion Date: 3rd November 2011

2 Assay performance

Rat uterine cytosol was collected, processed, and validated according to the OPPTS guideline. The limit of test chemical solubility was determined by visual observation. The competitive binding assay was performed using tubes containing 390 µL 'master mixture' (buffer + protease inhibitor + radioligand, 100 µL rat uterine cytosol (diluted to an appropriate protein concentration) and 10 µL of iprodione, positive control, weak positive control or negative control to give a final assay volume of 500 µL. Assay tubes were mixed by vortex and incubated for 16-20 hours at a temperature 4± 2ºC on a rotator. Following incubation, tubes were removed to an ice/water bath and 250 µL ice-cold hydroxyapatite slurry (60% in buffer) prior to mixing. Following the addition of a further 2 mL ice-cold buffer, the tubes were centrifuged for 10 minutes at 1000 x g. The resulting supernatant (containing unbound radioligand) was removed and discarded; a further 2 mL ice-cold buffer was added to the pellet which was resuspended and the centrifugation step repeated. Approximately 1.5 mL ethanol was added to each tube and left for 15-20 minutes with occasional mixing. The tubes were then centrifuged at 1000 x g for 10 minutes, 1 mL of the resulting supernatant was removed and assessed for radioactivity using liquid scintillation counting. For each data point, the non-specific binding value was subtracted; values were normalised to total binding and expressed as percentage specific binding.

3 Statistics

Values are expressed ad the mean and standard deviation for each assay run. Binding curves were fitted using non-linear regression according to the method of Hill (1910). IC50 vlaues were estimated using curve fitting software (GraphPad PRISM).

4 Assay acceptance criteria

The competitive binding assay was considered to be acceptable if all of the following criteria specified in the test guideline (OPPTS 890.1250) were met.

- Increasing concentrations of unlabeled 17β-oestradiol displaced the radioligand from the receptor in a manner consistent with one-site competitive binding.
- Ligand depletion was minimal.
- The parameter values (top, bottom, and slope) for 17β-oestradiol and the concurrent positive control (19-norethindrone) were within the tolerance bounds outlined in the OPPTS guideline.
- The solvent control did not alter the sensitivity or reliability of the assay.
- All tubes must have contained equal amounts of solvent.
- The negative control substance (octyltriethoxysilane) did not displace more than 25% of the radioligand from the ER on average across all concentrations.
- The test chemical was tested over a concentration range that fully defined the top of the curve (i.e. a range that showed that a top plateau was achieved), and the top was within 25 percentage points of either the solvent control or the value for the lowest concentration of the oestradiol standard for that run.

Classification of a chemical as a binder or non-binder was made on the basis of the average results of the three independent assay runs, each of which met the performance criteria and were consistent with each other. Each assay run was classified as either interacting, not interacting, equivocal or equivocal up to the limit of the tested concentrations. An assay run was classified as interactive if the lowest point on the fitted response curve within the range of the data was less than 50%. An assay run was classified as equivocal up to the limit of concentrations tested if there were no data points at or above a concentration of 10^{-6} M, and either a binding curve could be fitted, but 50% or less of the radioligand was displaced by a concentration of 10^{-6} M; or if a binding curve could not be fitted and lowest average binding among the concentration groups in the data exceeded 50%. An assay run was classified as not interactive if there were usable data points at or above 10^{-6} M, and either the lowest point on the fitted response curve within the range of the data exceeded 75%; or a binding curve could not be fitted and the lowest average binding among the concentration groups in the data exceeded 75%. An assay run was classified as equivocal if it fell in none of the categories above.

II. RESULTS AND DISCUSSION

A SOLUBILITY

Based on the results of an initial solubility assessment, iprodione was tested at final concentrations of 10^{-11} to 10^{-4} M in all three independent assay runs.

B ASSAY ACCEPTANCE

In all three independent assay runs of the assay, increasing concentrations of unlabeled 17β-oestradiol were shown to displace the radioligand (^{3}H-17β-estradiol) from the oestrogen receptor in a manner consistent with one-site competitive binding. Ligand depletion was below 15%. The assay solvent did not affect the sensitivity or reliability of the assay. Data were within acceptable ranges, with the following two exceptions:

- In the third assay run, the top plateau level for 19-norethindrone was marginally less than the specified range (89% compared to the specified range of 90%-110%).
- In the third assay run, the Hill Slope for 19-norethindrone was marginally less than the specified range (-1.2 compared to the specified range of –0.7 to -1.1).

These two deviations are considered to be minor in nature and do not reflect true deviation from the suggested ranges outlined in the OPPTS guideline. Therefore, all three independent runs of the assay are considered to have met the assay acceptance criteria and are concluded to be valid.

C RECEPTOR BINDING ASSAY

Four independent runs of the competitive binding assay were conducted, due to data for the positive and weak positive controls in the first run not meeting the acceptance criteria. The final concentrations of the iprodione assessed in the valid binding assays were: 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, $10^{-}7$, 10^{-6}, 10^{-5} and 10^{-4} M for all three valid independent assay runs. In all three of the valid assay runs, the mean specific binding was >84% for all concentrations of the negative control (octyltriethoxysilane); in two cases binding lay outside this range (44.4% and 41.5%, respectively) at the highest concentration. The report states that this phenomenon occurs occasionally and is usually accompanied by precipitation. Although precipitation was not specifically recorded in this study, the report notes that precipitation is difficult to detect by visual assessment as the assay tubes contain an opaque slurry.

In the first valid assay run, mean specific binding of iprodione was >87% at every soluble concentration, classifying it as non-interacting for this run. The weak positive control (19-norethindrone) showed a LogIC50 of -5.5 M; the LogIC50 of 17β-estradiol was -9.1 M. In the second valid assay run, mean specific binding of iprodione was >95% at every soluble concentration, classifying it as non-interacting for this run. The weak positive control had a LogIC50 of -5.5 M while the LogIC50 of 17β-estradiol was -9.0 M. In the third valid assay run, mean specific binding of ≥87% was noted for all concentrations of iprodione, classifying it as non-interacting for this run. The weak positive control had a LogIC50 of -5.4 M while the LogIC50 of 17β-estradiol was -8.9 M. The mean relative binding affinity (RBA) was 0.6 for 19-norethindrone.

Table 5.8.2-12: Oestrogen receptor binding assay results

Material	Concentration (M)	Specific binding (%)		
		Run 1	Run 2	Run 3
iprodione	10^{-4}	91.1 ±3.0	97.3 ±3.3	93.7 ±1.3
	10^{-5}	93.2 ±1.5	98.4 ±4.2	87.0 ±3.6
	10^{-6}	96.5 ±2.5	95.9 ±0.7	90.5 ±2.4
	10^{-7}	92.4 ±1.2	96.9 ±1.9	91.2 ±2.1
	10^{-8}	93.6 ±1.9	98.7 ±5.0	92.3 ±0.9
	10^{-9}	87.3 ±1.9	100.8 ±10.5	89.8 ±3.4
	10^{-10}	90.8 ±1.8	97.2 ±1.4	89.0 ±4.3
	10^{-11}	94.6 ±1.6	98.8 ±2.8	94.6 ±6.9
Oestradiol (NSB)	10^{-7}	0.0 ±0.3	0.0 ±0.4	0.0 ±0.1
	10^{-8}	6.4 ±0.5	9.0 ±1.6	8.5 ±1.1
	$10^{-8.5}$	19.8 ±0.5	24.8 ±1.1	25.1 ±0.7
	10^{-9}	43.2 ±2.4	46.8 ±2.7	54.0 ±8.3
	$10^{-9.5}$	69.1 ±2.2	74.7 ±1.6	70.9 ±3.1
	10^{-10}	83.5 ±2.5	87.3 ±4.6	84.7 ±1.9
	10^{-11}	94.7 ±1.5	96.0 ±2.0	93.9 ±1.6
19-Norethindrone	10^{-4}	2.0 ±0.1	1.7 ±1.4	1.9 ±0.0
	$10^{-4.5}$	8.0 ±0.4	5.8 ±0.4	8.7 ±0.8
	$10^{-5.5}$	46.4 ±3.1	49.4 ±3.3	48.8 ±0.5
	10^{-6}	71.4 ±0.8	73.2 ±2.3	72.3 ±1.3
	$10^{-6.5}$	88.3 ±0.2	90.2 ±1.8	85.1 ±1.5
	10^{-7}	92.2 ±2.5	97.0 ±3.7	91.0 ±1.1
	$10^{-7.5}$	90.3 ± 3.9	92.9 ±5.0	89.0±6.7
	$10^{-8.5}$	95.1 ±2.7	98.7 ±3.2	84.9 ±2.7
Octyltriethoxysilane	10^{-3}	44.4 ±1.0	41.5 ±2.0	91.0±1.8
	10^{-4}	85.4 ±2.3	84.8 ±3.1	91.9 ±0.8
	10^{-5}	95.5 ±4.1	101.3 ±4.0	91.3 ±3.2
	10^{-6}	89.6 ±1.9	92.9 ±2.6	90.0 ±4.2
	10^{-7}	91.5 ±3.2	97.6 ±2.8	86.8 ±1.7
	10^{-8}	95.1 ±0.5	95.1 ±2.8	92.2 ±2.7
	10^{-9}	91.9 ±2.2	95.8 ±4.6	92.7 ±3.0
	10^{-10}	91.5 ±0.2	99.9 ±4.0	94.5 ±3.5

E DISCUSSION

All independent runs of the assay were considered to have met the assay acceptance criteria. iprodione was classified as non-interacting in all three valid independent assay runs.

III. CONCLUSION

iprodione was classified as non-interacting in all three valid independent runs and is therefore classified as non-interacting with the oestrogen receptor according to EDSP guideline OPPTS 890.1250.

Report: CA 5.8.2/13
Wilga P.C., 2011a
Iprodione: Human recombinant aromatase assay
2011/1294833
Guidelines: EPA 890.1200
GLP: yes
(certified by United States Environmental Protection Agency)
[see 2014/1049126 Shearer K.S. 2014 c]

Executive Summary

The ability of iprodione to act as an inhibitor of aromatase activity was assessed using human CYP19 (aromatase) and P450 reductase Supersomes, with tritiated androstenedione as the aromatase substrate. Concentrations of iprodione tested were 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M; higher concentrations of iprodione were found not to be soluble in the assay system. Three independent assay runs were performed; in each assay each iprodione concentration was tested in triplicate. A positive control substance (4-hydroxyandrostenedione) was included in each assay run. Exposure to iprodione at the highest soluble test concentration resulted in mean aromatase activity of 82.4±0.8%. According to the data interpretation procedure outlined by the EPA for aromatase inhibition (conversion of androstenedione to oestrone, iprodione was classified as a non-inhibitor of aromatase. The positive control substance 4-hydroxyandrostenedione (Formestane) confirmed the sensitivity of the assay.

I. MATERIAL AND METHODS

A. MATERIALS

1 Test Material:

Description:	iprodione (powder)
Lot/Batch no.:	AE F062470-01-08 / SIN0000217
Purity:	98.1%
CAS:	36734-19-7
Stability:	Verified by Sponsor

2 Vehicle: DMSO

3 Reference compounds: Assay substrate: androstenedione (99.8% purity); ^{3}H-androstenedione (>97% radiopurity)

Positive control 4-hydroxyandrostenedione; 4-OH ASDN; Formestane (99.6% purity)

4 Test system	Human recombinant microsomes (Gentest, USA); consisting human CYP19 (Aromatase) and P450 reductase Supersomes. Assay Conditions: Microsomal protein 0.004 mg/mL NADPH 0.3 mM ^{3}H-androstenedione 100 nM Propylene glycol 5% Incubation time 15 minutes

B. STUDY DESIGN AND METHODS

1 Study dates

Study Initiation Date: 11 July 2011
Study Completion Date: 20 December 2011

2 Assay performance

Test substances were dissolved in DMSO (1% of the total assay volume). Fresh dilutions of the stock solution of test substances were prepared on the day of use such that the target concentration was achieved by the addition of 20 µL of the appropriate dilution to a 2 mL total assay volume. The assay buffer was 0.1 M sodium phosphate buffer, pH 7.4. NADPH (β-nicotinamide adenine dinucleotide phosphate, reduced form tetrasodium salt) was added as the required co-factor for CYP19; a stock solution of 6 mM NADPH was prepared in assay buffer and the final concentration in the assay was 0.3 mM. Assays were performed in 100 mm test tubes maintained at 37 ±2°C in a shaking water bath. Propylene glycol (5%), ^{3}H-androstenedione, NADPH and assay buffer were combined in the assay tubes with or without the test substance or the positive control at a total volume of 1 mL. The tubes and microsomal suspensions were placed at 37 ±2°C in the water bath for approximately 5 minutes, after which the assay was initiated by the addition of 1 mL of the microsomal suspension. The total assay volume was 2 mL. Tubes were then incubated for approximately 15 minutes at 37 ±2°C. Reactions were terminated by the addition of 2 mL of ice-cold methylene chloride, vortex-mixed for approximately 5 seconds and placed on ice. The tubes were then vortex-mixed again for an additional 20 to 25 seconds to extract residual androstenedione. The methylene chloride layer was removed and discarded and the aqueous layer was extracted twice more. Two 0.5 mL aliquots of the aqueous layer were transferred to duplicate liquid scintillation vials containing 10 mL liquid scintillation cocktail, mixed and samples analysed for radioactivity by liquid scintillation counting. Radioactivity found in the aqueous phase is due to the presence of tritiated water formed from the conversion of ^{3}H-androstenedione to oestrone by aromatase. Concentrations of iprodione tested in this assay were 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8},10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M; higher concentrations of iprodione were found not to be soluble in the assay system. Three independent assay runs were performed; in each assay each iprodione concentration was tested in triplicate. Solubility/precipitation of iprodione in the assay buffer was assessed visually in all assay runs. A positive control substance (4-hydroxyandrostenedione) was included in each assay run. Full activity controls (all assay components and vehicle (DMSO)) and background activity controls (all assay components except NADPH, and vehicle (DMSO)) were also included in the study design.

3 Statistics

Response curves were fitted by weighted least squares nonlinear regression analysis with weights equal to 1/Y. Model fits were carried out using a four-parameter regression model (XLfit; IDBS; Version 5.2.0.0; Fit Model 208) and Tukey's Bi-Weight statistical analysis for outlier analysis.

4 Assay acceptance criteria

Data from this assay are used to classify a test substance according to the measured ability to inhibit aromatase activity. To be classified as an inhibitor, the data must fit the four-parameter regression model to yield an inhibition curve and result in greater than 50% inhibition at the highest concentration tested.

II. RESULTS AND DISCUSSION

A SOLUBILITY

iprodione at a concentration of 10^{-3} M was tested in Run 1 but was not included in the final analysis due to precipitation observed at this concentration. iprodione was tested at final concentrations of 10^{-11} to 10^{-4} M.

B ASSAY ACCEPTANCE

In three independent runs of the assay, the average of the background activity controls (NSB) was approximately 0% and was therefore within the acceptable range of -5 to +6%. The average of the activity controls (TA) within a run was approximately 100% and was therefore within the acceptable range of 90-110%). The mean background activity controls were ≤ 15% of the full activity controls, the limit established in the guideline. The mean aromatase activity in the full activity control samples was 0.598 nmol/mg-protein/min and was therefore above the minimum activity of 0.100 nmol/mg-protein/min minimum defined in the study guideline. All independent runs of the assay were therefore considered to have met the assay acceptance criteria and are considered to be valid and definitive.

C AROMATASE INHIBITION ASSAY

Activity was expressed as a percentage of the full activity control. Evidence for the slight inhibition of aromatase by iprodione was seen in all runs at the highest soluble concentration of 10^{-4} M. Mean aromatase activity after treatment with iprodione at the highest soluble concentration of 10^{-4} M over the three assay runs was calculated to be 82.4 ±0.8% of the control activity. The coefficient of variation for each concentration of iprodione was within the 15% specified in the test guideline.

Table 5.8.2-13: Inhibition of aromatase activity by iprodione

iprodione Concentration (M)	Run 1	Run 2	Run 3
TA	99.52 ±1.283	98.41±1.918	99.1 2 ±0.906
NSB	-0.03 ±0.044	0.02 ±0.059	-0.01 ±0.005
10^{-3}	74.86 ±2.612*		
10^{-4}	82.19 ±1.568	83.22 ±2.952	81.74 ±0.940
10^{-5}	94.95 ±1.016	99.44 ±5.081	97.49 ±1.259
10^{-6}	98.45 ±1.444	102.81 ±1.553	92.52 ±2.746
10^{-7}	98.95 ±1.093	103.63 ±0.784	100.21 ±0.968
10^{-8}	100.09 ±3.629	103.62 ±2.226	99.89 ±0.934
10^{-9}	99.70 ±3.295	101.77 ±1.291	98.09 ±1.509
10^{-10}	103.27 ±0.741	86.94 ±13.486	98.87 ±0.497
10^{-11}		97.20 ±7.610	97.32 ±1.736

**concentration exceeding the limit of solubility; not included in subsequent analyses*
TA: Full activity control
NSB: Background activity control

D POSITIVE CONTROL ASSAY

Aromatase inhibition by the positive control substance 4-hydroxyandrostenedione was seen in all assay runs, thereby demonstrating the sensitivity of the assay. The coefficient of variation for each concentration of the positive control was within the 15% specified in the test guideline.

Table 5.8.2-14: Inhibition of aromatase activity by the positive control

4-OH ASDN Concentration (M)	Run 1	Run 2	Run 3
TA	100.48 ±2.612	101.59 ±0.650	100.88 ±2.492
NSB	0.03 ±0.025	-0.02 ±0.035	0.01 ±0.002
10^{-5}	0.72 ±0.037	0.75 ±0.100	0.76 ±0.073
10^{-6}	5.99 ±0.243	6.11 ±0.288	5.94 ±0.085
$10^{-6.5}$	16.20 ±0.394	16.02 ±0.806	15.97 ±0.477
10^{-7}	35.05 ±0.978	36.64 ±0.194	34.41 ±0.412
$10^{-7.5}$	61.66 ±0.761	63.12 ±2.977	61.76 ±0.112
10^{-8}	83.25 ±0.531	83.55 ±1.771	82.65 ±2.431
10^{-9}	96.75 ±2.533	96.23 ±0.004	93.47 ±2.135
10^{-10}	100.09 ±2.626	104.73 ±0.574	96.62 ±1.217

E DISCUSSION

All independent runs of the assay were considered to have met the assay acceptance criteria. Mean aromatase inhibition by iprodione at the highest soluble concentration of 10^{-4} M over the three assay runs was calculated to be 82.4 ±0.8% of the control activity.

III. CONCLUSION

iprodione was determined to be a non-inhibitor of aromatase in this validated assay as defined by EDSP guideline OPPTS 890.1200.

Report:	CA 5.8.2/14 Ramirez Hernandez T., 2013a BAS 610 F (Iprodione) - Steroidogenesis assay (human cell line H295R) 2013/1188655
Guidelines:	EPA 890.1550, OECD 456 (28 July 2011)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The ability of iprodione to act as an inhibitor of androgen steroidogenesis was investigated in an assay *in vitro* using the H259R human adrenocortical carcinoma cell line. Concentrations of 1, 3, 10, 30 and 100 µM iprodione (dissolved in DMSO) were investigated in three separate assay runs, based on the results of an initial cytotoxicity assay. In the main experiment, slight cytotoxicity (as assessed by the MTT assay) was seen at iprodione concentrations of 30 and 100 µM. A level of cytotoxicity exceeding the 20% threshold was seen at the concentration of 100 µM iprodione in the first assay run. In the first assay, statistically significant reductions in testosterone concentration were seen at iprodione concentrations of 30 and 100 µM; however exposure to the highest concentration was associated with cytotoxicity (77% relative cell survival) and precipitation of the test material. In the second assay run, a significantly reduced testosterone concentration was seen only at the highest concentration of 100 µM. In the third assay run, significantly reduced testosterone concentrations were seen at iprodione concentrations of 30 and 100 µM. Precipitation of iprodione was also observed at the highest concentration of 100 µM. Exposure of cells to the positive control substance forskolin (an inducer of steroidogenesis) resulted in a marked increase in testosterone concentration and exposure of cells to the positive control prochloraz (an inhibitor of steroidogenesis) resulted in a marked reduction in testosterone concentration; thereby confirming the sensitivity of the assay. There is therefore some evidence for iprodione acting as an inhibitor of androgen steroidogenesis under the conditions of this *in vitro* screening assay.

I. MATERIAL AND METHODS

A. MATERIALS

1 Test Material:

Description:	BAS 610 F (iprodione)
Lot/Batch no.:	MD2085
Purity:	99.8%
CAS:	36734-19-7
Stability:	Stability of iprodione in the vehicle verified for 4 hours at room temperature. iprodione confirmed by the sponsor to be stable over the duration of the study

2 Vehicle: DMSO

3 Reference compounds: Positive controls:
Forskolin (1 µM, 10 µM)
Prochloraz (0.1 µM, 1 µM)
Negative control:
Culture medium (DMEM/F-12 with 2.5% serum)
Vehicle control:
DMSO (0.1%)

4 Test system H295R human adrenal cortex carcinoma cell line (ATCC, CRL-2128)

B. STUDY DESIGN AND METHODS

1 Study dates

Study Initiation Date: 26th April 2013
Study Completion Date: 16th July 2013

2 Assay performance

The H295R human adrenal cortex carcinoma cell line was obtained from the American Type Culture Collection (ATCC CRL-2128). Cells were cultured in maintenance medium consisting of Dulbecco's Modified Eagle's Medium and Ham's F-12 Nutrient mixture supplemented with 2.5% of Nu-Serum, 1% ITS Premix solution and 1% of penicillin/streptomycin antibiotic mixture. Cultures were incubated at 37°C in a 5% CO_2 atmosphere in 175 cm^2 cell culture flasks with 50 mL of cell culture medium. For the steroidogenesis experiments, cells (of passage number ≥3) were cultured in 24-well culture plates at a density of 2.5-3.0 x10^6/mL maintenance medium without antibiotics. Cells were cultured at 37°C in a 5% CO_2 atmosphere for 24 hours. After 24 hours, the culture medium was replaced with fresh maintenance medium without antibiotics and the test substance, vehicle or positive control. Cells were exposed for 48 hours to 0.1% DMSO (vehicle control), iprodione (concentrations of 1, 3, 10, 30 and 100 µM), forskolin (an inducer of steroidogenesis; 1 and 10 µM) or prochloraz (an inhibitor of steroidogenesis; 0.1 and 1 µM). After treatment, cells were assessed microscopically and cultures exhibiting over-confluence or marked cytotoxicity were discarded. The cell supernatants were removed and frozen prior to the analysis of testosterone levels. Cells were rinsed with PBS and cytotoxicity assessed using the MTT assay in experimental and in parallel quality control (QC) plates. Testosterone levels in the cell culture supernatant were measured by automated ELISA. Three independent experiments were performed.

3 Statistics

For data analyses, only non-cytotoxic concentrations of the test substance (≤20% cytotoxicity compared to vehicle control cultures) were evaluated. For evaluation of effects on hormone production, the results were normalised to the mean vehicle control value of each test plate, and results expressed as changes relative to the vehicle control in each test plate. Before calculating relative change, the background value (average BSA Buffer) was subtracted from all values. Cell viability data were expressed as cell viability relative to the average viability of the vehicle controls for each test plate. Additional statistical analyses were carried out according to the Welch t-test. Since the statistical power of three samples was poor, a Williams test analysis was also performed. Data assessments were only considered valid if based on at least two independent runs.

4 Assay acceptance and interpretation criteria

Acceptance criteria according to OECD 456 and OCSPP 890.1550 specify that hormone production in the vehicle control should be at least five times the loq of 0.3 nM testosterone; hormone production by cells exposed to the inducer forskolin (10 µM) should be at least 1.5 times the vehicle control; and that hormone production by cells exposed to the inhibitor prochloraz (1 µM) should be half or less that of the vehicle control. Cultures with cytotoxicity of 20% or greater and cultures with visible precipitation of the test material are not considered in the final assessment.

A chemical is judged to be positive in this assay if the induction or inhibition of hormone synthesis is statistically different ($p \leq 0.05$) from the vehicle control at two adjacent concentrations in at least two independent runs. A chemical is judged to be negative following two independent negative runs, or in three runs (comprising two negative runs and one equivocal or positive run). Results at concentrations exceeding the limits of solubility or at cytotoxic concentrations are not included in the interpretation of results.

II. RESULTS AND DISCUSSION

A SOLUBILITY AND CYTOTOXICITY

A range-finding assay identified that iprodione in 0.1% DMSO was soluble in the test system at concentrations of up to 1000 µM. Slight cytotoxicity was detected in a preliminary assay at concentrations of 30 µM and above. In the main steroidogenesis assay, slight cytotoxicity (as assessed by the MTT assay) and precipitation of iprodione were observed at the highest concentration of 100 µM

B ASSAY ACCEPTANCE

The assay met the acceptance criteria specified in OECD 456 and OCSPP 890.1550.

C STEROIDOGENESIS ASSAY

The effects of iprodione on testosterone synthesis were assessed in three independent experiments. In Experiment 1, a statistically significant reduction in testosterone concentration was seen at iprodione concentrations of 30 and 100 µM; cytotoxicity (77%) was seen at the highest concentration in this experiment. In Experiment 2, a reduction in testosterone concentration was seen at iprodione concentrations of 3-100 µM; values attained statistical significance only at 100 µM. In Experiment 3, a reduction in testosterone concentration was seen at iprodione concentrations of 30 and 100 µM; values attained statistical significance only at 100 µM. Exposure of cells to the positive control forskolin resulted in a marked increase in testosterone concentration; exposure of cells to the positive control prochloraz resulted in a marked reduction in testosterone concentration.

Table 5.8.2-15: Steroidogenesis assay: summary of results

Substance	Experiment 1		Experiment 2		Experiment 3	
	Testosterone	Viability	Testosterone	Viability	Testosterone	Viability
Untreated control	1.40±0.33	0.97±0.01	1.08±0.34	0.96±0.01	1.23±0.15	0.98±0.02
Vehicle control	1.00±0.04	1.00±0.01	1.00±0.13	1.00±0.00	1.00±0.04	1.00±0.01
iprodione (0 µM)	1.00±0.12	1.00±0.02	1.00±0.09	1.00±0.01	1.00±0.25	1.00±0.03
iprodione (1 µM)	1.10±0.17	1.00±0.01	1.14±0.22	1.02±0.03	0.95±0.14	1.03±0.01
iprodione (3 µM)	1.12±0.14	1.03±0.03	0.89±0.02	1.03±0.01	0.83±0.13	1.00±0.01
iprodione (10 µM)	1.04±0.08	1.01±0.00	0.92±0.05	1.01±0.03	0.84±0.11	1.00±0.01
iprodione (30 µM)	**0.76*±0.04**	0.92±0.01	0.83±0.17	0.94±0.01	**0.55**±0.03**	0.95±0.01
iprodione (100 µM)	**0.72*±0.15**	**0.77±0.07**	**0.74*±0.07**	0.84±0.01	**0.59**±0.11**	0.88±0.02
Forskolin (1 µM)	2.30±0.05	1.10±0.04	1.62±0.05	1.09±0.02	1.72±0.07	1.10±0.01
Forskolin (10 µM)	3.79±0.26	1.14±0.0.3	2.14±0.07	1.13±0.01	2.28±0.15	1.11±0.02
Prochloraz (0.1 µM)	0.73±0.08	1.00±0.01	0.64±0.05	0.99±0.01	0.69±0.07	1.01±0.01
Prochloraz (1 µM)	0.22±0.05	1.03±0.02	0.31±0.05	1.01±0.01	0.16±0.01	1.05±0.01

**significantly different to controls (p<0.05); **p<0.01*
shading indicates cytotoxicity of 20% or greater

E DISCUSSION

The experiment met the assay acceptance criteria. Evidence for a reduction in testosterone synthesis in the H295R cell line was seen at iprodione concentrations of 30 and 100 µM; cytotoxicity was seen at these concentrations, but only once (Experiment 1 at 100 µM did the level of cytotoxicity exceed 20%.

III. CONCLUSION

Evidence for the inhibition of testosterone synthesis was seen in this screening assay in the human H295R cell line at iprodione concentrations of 30 and 100 µM.

It is important to mention that in a second study (not reported), iprodione and plant metabolites where intended to be tested for impact on steroidogenesis under the same assay conditions. Iprodione was tested up to 45µM based on cytotoxicity assessment.In contrast to the first study with iprodione, the borderline positive results could not be replicated. Therefore the study was terminated preliminary without assessment of metabolites

CA 5.8.3 Endocrine disrupting properties

5.8.3.1 Introduction

Findings of potential relevance to endocrine disruption identified in repeated dose, investigative and mechanistic studies performed with iprodione are discussed in this section. A mode of action for the potential endocrine disrupting properties of iprodione is proposed, based on the findings of the studies discussed.

5.8.3.2 Overview of relevant effects seen in repeated dose toxicity studies

Repeated dose toxicity studies performed with iprodione in the rat, mouse and dog identify effects on organs and tissues of the endocrine system; effects are seen in both sexes at high doses. Findings are characterised by weight changes, gross and/or histopathological evidence of cellular hypoplasia, hypertrophy or hyperplasia in organs or tissues responsive to, and/or responsible for the production of steroid hormones. Some organs are affected by weight changes only, whereas others display macroscopic and/or histopathological effects secondary to the observed weight changes. Consistently, the most sensitive effects are those affecting the adrenal gland.

a) Short-term repeated dose studies in the mouse

In a 28-day mouse study (BASF DocID R014614) slight and reversible testicular interstitial cell hyperplasia at a dietary concentration of 9500 ppm is reported. A partial or total block of spermatogenesis is reported at the highest dose level of 15000 ppm iprodione; however this dietary concentration resulted in significant mortality and findings are noted only in decedent animals. Findings may not therefore represent a direct effect of iprodione on the testes or endocrine system. This assumption is further supported by results of the 90-day study in the mouse (BASF DocID C022886) where no effects on the testes up to the highest dietary concentration of 12000 ppm were identified. Increased adrenal weight accompanied by hypertrophy and vacuolisation of the *zona fasciculata* and *zona glomerulosa* are observed in both sexes at dietary concentrations of 6000 ppm and higher, and in females also at ≥ 1500 ppm. At ≥ 6000 ppm, uterine atrophy and an absence of *corpora lutea* is also observed in female mice. Relevant findings in the mouse studies are summarised in Table 5.8.3-1.

b) Short-term repeated dose studies in the rat

An older 90-day study in the rat (BASF DocID C022485) reports effects of iprodione on the adrenals (increased weight, hypertrophy and discoloration associated with lipid accumulation) at dietary concentrations of ≥ 1000 ppm. A more modern 90-day study (BASF DocID C022531) notes various effects at dietary concentrations of 2000 ppm (reduced ovary weight, vacuolisation of the adrenal *zona fasciculata*, enlargement of the adrenal *zona glomerulosa*, uterine atrophy, reduced numbers of *corpora lutea* and prostate atrophy) and 3000 ppm (additionally reduced uterus weight, testicular interstitial cell hyperplasia and reduced seminal vesicle secretion). In the most recent 90-day rat study (BASF DocID R014676), increased adrenal weight accompanied by vacuolisation and hypertrophy of the *zona fasciculat*a, vacuolisation of the *zona glomerulosa,* atrophy of the seminiferous tubules, reduced prostate weight and secretion were all observed at the highest dietary concentration of 3000 ppm. Vacuolisation of the adrenal *zona fasciculat*a and *zona glomerulosa* was also seen at a dietary concentration of 800 ppm; a dose level at which effects on other organs or tissues were absent. Relevant findings in the rat studies are summarised in Table 5.8.3-1.

c) Short-term repeated dose studies in the dog

An older 90-day study in the dog (BASF DocID C021584) reports reduced testes weight, testicular hypoplasia and prostatic hypoplasia in one male at a dietary dose level of 7200 ppm. Prostatic hypoplasia is also reported in one dog at 800 ppm. Findings at the highest dose level are associated with bodyweight effects and consequently not represent a specific organ effect of iprodione.

d) Overview of short-term repeated dose toxicity studies

Repeated dose toxicity studies performed with iprodione in the rat, mouse and dog consistently identify effects on organs and tissues sensitive to steroid hormones and/or involved in steroid hormone synthesis (e.g. adrenals, testis, prostate, ovary and uterus).

In the rat, data indicate that following short-term exposure the adrenal is the most sensitive target organ of iprodione. Effects on the adrenals included increased organ weight, enlargement and cellular hyperplasia in areas of the adrenal cortex responsible for steroid hormone synthesis. No information is given in the study report on the grading of adrenal vacuolation; therefore it is not possible to discriminate the adrenal effects as either adverse or solely treatment related. In a recent 2-generation study in rat, adrenal vacuolation was observed and regarded as non-adverse. Assuming identical mode of action in both studies, the same interpretation (non-adverse) can therefore be applied to the findings in the 90-day study in rat. The lowest endocrine-related no effect level in the short-term rat studies is 500 ppm (equivalent to mean achieved intakes of 31 and 36 mg/kg bw/d in males and females respectively) (BASF DocID R014676), based on adrenal histopathology at 800 ppm (equivalent to mean intakes of about 49 and 59 mg/kg bw/d) in males and females respectively.

The lowest endocrine-related effect level seen in the mouse is 1500 ppm (equivalent to approximately 300 mg/kg bw/d) from the 90-day study (BASF DocID C022886), based on adrenal weight increase in addition to hypertrophy/vacuolation of the *zona fasciculata*. The mouse is therefore shown to be less sensitive to the endocrine-related effects of iprodione than the rat; however the short-term toxicity studies in the mouse also identify the adrenals as the most sensitive target of iprodione.

An indication of reduced prostate weight was seen in a 90-day dog study at dietary dose levels of 800 ppm (equivalent to 30 mg/kg bw/d) and higher; however the interpretation of effects on prostate weight in the dog is problematic due to the high level of background variability and the influence of age and bodyweight on this parameter. Prostate effects are seen inconsistently in 12-month dogs studies (described below).

Data from short-term repeated dose toxicity studies performed with iprodione therefore show the rat and dog of comparable sensitivity, with overall endocrine-related NOELs of 31-36 mg/kg bw/d and 30 mg/kg bw/d respectively with about 50-60 mg/kg bw/d being the rat LOEL; an overall endocrine-related LOEL of approximately 300 mg/kg bw/d is identified for the mouse, which is identified as the less sensitive species.

Table 5.8.3-1: Endocrine-related effects in short-term repeated dose toxicity studies with iprodione

Study (Batch / purity)	Endpoint	Dose level	LO(A)EL ppm [mg/kg bw/d]	NO(A)EL ppm [mg/kg bw/d]	Reference
Mouse 28-day (MAG 449 / unknown)	Partial/total block of spermatogenesis considered to be secondary to general excessive toxicity	15000 ppm	9500 ppm [1400 mg/kg bw/d]	6000 ppm [830 mg/kg bw/d]	[redacted] (1979) BASF DocID R014614
	Leydig Cell hyperplasia	9500 ppm			
Mouse 90-day (DA 604 / 95.7%)	Uterus atrophy	≥ 6000 ppm	1500 ppm [327 mg/kg bw/d]	< 1500 ppm [327 mg/kg bw/d]	[redacted] (1990) BASF DocID C022886
	Reduced *corpora lutea*	≥ 6000 ppm			
	Adrenals (Zona fasciculata vacuolation/ hypertrophy)	12000 ppm (M,F) ≥ 1500 ppm (F)			
	Adrenals (Zona glomerulosa vacuolation/ hypertrophy)	12000 ppm (M,F) ≥ 6000 ppm (F)			
	Increased adrenal weight	12000 ppm (M,F) ≥ 1500 ppm (F)			
Rat 90-day (MAG 484 / unknown)	Increased adrenal weights, discoloration, swelling, lipid deposition	≥ 1000 ppm	1000 ppm	300 ppm	Itabashi *et al* (1978) BASF DocID C022485
Rat 90-day (DA 604 / 95.7%)	Uterus: weight reduced	3000 ppm	2000 ppm [≈200 mg/kg bw/d]	1000 ppm [≈100 mg/kg bw/d]	[redacted] (1990) BASF DocID C022531 [redacted] (1993) BASF DocID C025337
	Testes: Interstitial cell hyperplasia;				
	Seminal vesicle: reduced secretion				
	Reduced ovary weight	≥ 2000ppm			
	Adrenal: *Zona fasciculata* vacuolation *Zona glomerulosa* enlargement				
	Uterus: atrophy				
	Corpora lutea: reduced number				
	Prostate: atrophy				

Study (Batch / purity)	Endpoint	Dose level	LO(A)EL ppm [mg/kg bw/d]	NO(A)EL ppm [mg/kg bw/d]	Reference
Rat 90-day (9426801 / 97.1%)	Adrenal: weight increased; *Zona fasciculata* vacuolation/hypertrophy; *Zona glomerulosa* enlargement	3000 ppm	800 ppm [≈50 mg/kg bw/d]	500 ppm [31 mg/kg bw/d]	[redacted] (1997) Continuous dosing BASF DocID R014676
	Seminiferous tubule atrophy				
	Prostate: weight and secretion reduced				
	Adrenal: corticocellular vacuolation	800 ppm			
Dog 90-day (GD 5740 / 100%)	Testes: weight reduced; testicular hypoplasia; prostatic hypoplasia (questionable)	7200 ppm	800 ppm [≈30 mg/kg bw/d]	<800 ppm [≈30 mg/kg bw/d]	[redacted], 1973 BASF DocID C021584
	Prostatic hypoplasia (questionable)	800 ppm			

e) Chronic toxicity and carcinogenicity studies

Chronic exposure to iprodione in the rat (BASF DocID R014586) causes a number of potentially endocrine related effects in male and female reproductive organs and the adrenals. Increased testes weight, testicular atrophy, testicular interstitial cell hyperplasia, reduced epididymal sperm; increased adrenal weight, enlargement and vacuolisation of the *zona glomerulosa*, *zona fasciculata* and *zona reticularis*; small prostate and prostatic atrophy; small seminal vesicles with reduced contents and thickened uterus are seen at the highest dietary concentration of 1600 ppm (82 mg/kg bw/d). A smaller number of findings (small prostate, testicular interstitial cell hyperplasia, reduced epididymal sperm, enlargement and vacuolisation of the *zona glomerulosa*, *zona fasciculata* and *zona reticularis*) is also observed at a dietary concentration of 300 ppm (15 mg/kg bw/d). The incidence of testicular interstitial cell tumours is significantly elevated at a dietary concentration of 1600 ppm, the highest dose tested.

In the mouse carcinogenicity study (BASF DocID C021564), effects on various organs (reduced ovary and uterus weights, small testes, testicular interstitial cell hypertrophy and vacuolisation, reduced *corpora lutea*, luteinisation of ovarian interstitial cells, increased adrenal weights in both sexes and *zona fasciculata* cellular hypertrophy in females) were apparent at a dietary concentration of 4000 ppm (600 mg/kg bw/d); testicular interstitial cell hypertrophy and vacuolisation were also observed at a dietary concentration of 800 ppm (125 mg/kg bw/d). A slightly increased incidence of ovarian luteoma was seen at the highest dietary concentration of 4000 ppm. Subsequent analysis of this finding by a Pathology Working Group (BASF DocID B003580) showed that the luteoma incidence lies within the historical control range and does not attain statistical significance.

A 12-month dog study (BASF DocID C021579) reports increased adrenal weight and reduced prostate weight at a dietary concentration of 3600 ppm (150mg/kg bw/d); reduced prostate weight and histopathological changes in the adrenals (enlargement of the *zona fasciculata* and *zona glomerulosa*, associated with cellular hyperplasia) were observed at this dose level and also at 600 ppm (25 mg/kg bw/d). In contrast, a more recent 12-month study (BASF DocID C021573), performed to clarify the effects seen in the earlier study, does not show any effects of treatment at the single dietary concentration of 600 ppm (25 mg/kg bw/d). An overall NOEL of 600 ppm (equivalent to mean achieved intakes of approximately 25 and 26 mg/kg bw/d in males and females respectively) can therefore be derived for the dog.

Table 5.8.3-2: Endocrine-related effects in long-term repeated dose toxicity studies with iprodione

Study (Batch / purity)	Endpoint	Dose level	LO(A)EL ppm [mg/kg bw/d]	NO(A)EL ppm [mg/kg bw/d]	Reference
Rat 2-year (DA 604 / 95.7%)	Prostate: atrophy	1600 ppm	300 ppm [≈15 mg/kg bw/d]	150 ppm [≈7.5 mg/kg bw/d]	[redacted] (1992) BASF DocID R014586
	Adrenal: weight increased; *Zona glomerulosa* enlargement *(interim)*				
	Testes: weight increased				
	Seminiferous tubules: atrophy				
	Seminal vesicles small and reduced contents				
	Uterus: thickend				
	Prostate: small	≥ 300ppm			
	Adrenal: *zonae glomerulosa/fasciculata/ reticularis* enlargement/vacuolation (termial) *Zona fasciculata* vacuolation (interim)				
	Tested: interstitial cell hyperplasia *(in historical control range (see chapter MCA 5.5)*				
	Seminal vesicle reduced secretion *(in historical control range (see chapter MCA 5.5)*				
	Epididymides reduced sperm *(at the limit of historical control range (see chapter MCA 5.5)*				
Mouse carcinogenicity (DA 604 / 95.7%)	Ovary: weight reduced	4000ppm	800 ppm [≈125 mg/kg bw/d]	160 ppm [≈25 mg/kg bw/d]	[redacted] (1993) BASF DocID C021564 [redacted] (2001) BASF DocID B003580
	Uterus: weight reduced				
	Testes: small				
	Adrenal*: Zona fasciculata* enlargement (interim)				
	Ovary: interstitial cell luteinisation				
	Corpora lutea: reduced				
	Testes: interstitial cell hypertrophy, vacuolation	≥ 800 ppm			
Dog 12-month (DA 237 / 96.5%)	Increased adrenal weight	3600 ppm	600 ppm [≈25 mg/kg bw/d]	100 ppm [4.2 mg/kg bw/d]	[redacted] (1985) BASF DocID C021579
	Prostate: weight reduced	≥ 600ppm			
	Adrenal: cell changes				
Dog 12-month (8906201 / 96.2%)	-	-	>600 ppm [≈25 mg/kg bw/d]	600 ppm [≈25 mg/kg bw/d]	[redacted] (1991) BASF DocID C021573

5.8.3.3 Information from reproductive toxicity studies

The reproductive toxicity of iprodione has been investigated in a number of studies in the rat, although the value of the older studies is somewhat limited by deficiencies in study design and/or reporting. In an older three generation study (BASF Doc ID C022999), no clear effects were observed on fertility or reproductive performance in this study, which was performed at dietary concentrations of up to 2000 ppm (120 mg/kg bw/d). A reduction in mean litter size seen in the F_2 generation was not seen in the F_1 or F_3 generations and is therefore not considered to be related to treatment. In a subsequent two-generation study (BASF DocID C021572) performed at dietary concentrations of up to 3000 ppm (200 mg/kg bw/d), no effects were noted on fertility or reproductive performance. A reduction in post-natal pup survival was seen in one generation; however effects are associated with maternal toxicity. In the most recent study (BASF DocID 2013/1251918), conducted according to OECD TG 416 (2001) including enhanced parameters, no effects of treatment were seen on fertility or reproductive performance at dietary concentrations of up to 1500 ppm (156 mg/kg bw/d). The more extensive investigations performed as part of this study revealed nipple present in male pups at PND 20 at the highest dose level of 1500 ppm. Nipples were also evident in a single animal at 750 ppm (66 mg/kg bw/d). Delayed sexual maturation in male offspring was observed at 750 and 1500 ppm. The effects on sperm morphology from low-dose on were regarded not treatment related but within the concurrent and/or historical control range. In addition, a treatment related but non-adverse increase in adrenal vacuolation of the *zonae fasciculata* and *glomerulosa* was observed in males at 1500 ppm and females at ≥ 750 ppm.

The results of the reproductive studies do not therefore identify any effects of iprodione on fertility. The most sensitive parameters identified in the recent two-generation study relate to the development of male offspring and adrenal vacuolation; the overall reproductive NOAEL is therefore 300 ppm (32 mg/kg bw/d) based on a delay in male sexual maturation seen at the LOAEL of 750 ppm (82 mg/kg bw/d).

Table 5.8.3-3: Endocrine-related effects in reproductive toxicity studies with iprodione

Study (Batch / purity)	Endpoint	Dose level	LO(A)EL ppm [mg/kg bw/d]	NO(A)EL ppm [mg/kg bw/d]	Reference
Rat multi-generation	Reduced litter size (F2) only	2000 ppm	2000 ppm [≈120 mg/kg bw/d]	500 ppm [≈30 mg/kg bw/d]	Coquet (1976) BASF DocID C022999
Rat multi-generation (8906201 / 96.2%)	Maternal toxicity, reduced litter size	2000 ppm	2000 ppm [≈200 mg/kg bw/d]	1000 ppm [≈100 mg/kg bw/d]	Henwood (1991) BASF DocID C021572
Rat multi-generation (COD-001260 / 97.8%)	Nipple retention (M)	1500 ppm	750 ppm [82 mg/kg bw/d]	300 ppm [32 mg/kg bw/d)	[redacted] (2013) BASF DocID 2013/1251918
	Delayed puberty (M) Adrenal vacuolation of *zonae fasciculata* and *glomerulosa* (M,F)	≥ 750 ppm			

5.8.3.4 Information from investigative studies

The potential endocrine effects of iprodione indicated by the results of the standard repeated dose toxicity studies have been further investigated in a number of screening and mechanistic studies *in vitro* and *in vivo*. The results of these studies are summarised below.

a) Information from investigative studies *in vivo*

In the original dossier from 1997 a mechanistic study reports that dietary administration of iprodione to male rats at a dietary concentration of 3000 ppm for 2, 7 or 1 day(s) resulted in increased adrenal and testis weight and reduced weights of the accessory sex organs (BASF DocIDs C024293). This study did not show any effect of treatment on circulating testosterone levels. Blystone *et al* (2007), however, demonstrated a clear reduction in the levels of circulating testosterone in male rats administered iprodione by gavage at dose levels of 50, 100 and 200 mg/kg bw/d from Day 23 *post partum*. Progesterone levels were unaffected by treatment and other hormone measurements were largely below the limit of detection and not clearly affected by treatment. Findings in this study were associated with a delay in balano-preputial separation; increased adrenal weights, reduced seminal vesicle and epididymal weights were also seen at the highest dose level. No effects were seen at the lowest dose level of 50 mg/kg bw/d. Two studies (BASF DocID B003656, BASF DocID C019327) similarly demonstrated a reduction in circulating testosterone levels and an increase in the level of circulating luteinising hormone (LH) in male rats administered iprodione by gavage at dose levels of 70 and 300 mg/kg bw/d. The fall in testosterone level was rapid (seen within 0.5 hours of dosing), and transient (the effect was reversible within 4-6 hours). The reduction in circulating testosterone was accompanied by a similarly rapid and transient increase in the levels of circulating LH. In a further study investigating hormonal effects (BASF DocID C024675), circulating testosterone levels in sexually mature male Sprague-Dawley rats were significantly reduced at 300 mg/kg bw/d (1-4 hours after dosing) and at 70 mg/kg bw/d (at 2 hours). Testosterone levels had returned to normal within 24 hours (300 mg/kg bw/d) or 4 hours (70 mg/kg bw/d). Circulating LH levels were significantly higher at 70 and 300 mg/kg bw/d at 4 hours and had recovered by 24 hours. No effects on testosterone or LH levels were observed at a dose level of 6 mg/kg bw/d iprodione. Blystone *et al* (2009) reports In a Hershberger Assay a reduction in the weights of secondary sexual tissues in castrated male rats administered testosterone propionate by injection and iprodione at gavage dose levels of ≥50 mg/kg bw/d for 10-11 days. Prostate weight was significantly reduced at 200 mg/kg bw/d, LABC weight was significantly reduced at dose levels of ≥50 mg/kg bw/d; adrenal weight was significantly increased at 200 mg/kg bw/d. Circulating testosterone and LH levels were unaffected by treatment in this study.

A study (BASF DocID R014642) observed that gavage administration of 300 mg/kg bw/d iprodione to male rats for 30 days did not result in visually detectable proliferation of testicular interstitial cells, but did result in increased in PCNA labelling. Another study (BASF DocID C025273) also demonstrated that administration of iprodione at gavage dose levels of 70 and 300 mg/kg bw/d on 14 consecutive days caused a statistically significant and dose-related increase in testicular interstitial cell proliferation as assessed using BrdU labelling. No effect was seen at a dose level of 6 mg/kg bw/d; thereby demonstrating a clear threshold for this effect.

Iprodione at dose levels of 200 and 400 mg/kg bw was not shown to have uterotrophic activity in the immature female rat assay (BASF DocID 2012/1364406), indicating a lack of oestrogenic activity in this model. However a delay in the time to first oestrus and a delay in sexual maturation (time to vaginal patency) were seen in a female pubertal assay (BASF DocID 2012/1364405) at a dose level of 300 mg/kg bw (associated with bodyweight effects) but not at a dose level of 150 mg/kg bw/d.

Table 5.8.3-4: Summary of investigative studies *in vivo*

Study (Batch / purity)	Endpoint	Dose level	LO(A)EL ppm [mg/kg bw/d]	NO(A)EL ppm [mg/kg bw/d]	Reference
Male pubertal assay (MO07526JO / 97%)	Increased adrenal weight	200 mg/kg bw/d	50 mg/kg bw/d	<50 mg/kg bw/d	Blystone et *al* (2007a) BASF DocID 2008/1101757
	Reduced seminal vesicle weight				
	Reduced epididymal weight				
	Delayed sexual maturation	≥ 100 mg/kg bw/d			
	Reduced circulating testosterone	≥ 50 mg/kg bw/d			
Hershberger assay (MO07526JO / 97%)	Reduced prostate weight	200 mg/kg bw/d	50 mg/kg bw/d	<50 mg/kg bw/d	Blystone *et al* (2009a) BASF DocID 2009/1130622
	Reduced LABC weight	≥ 50 mg/kg bw/d			
Plasma testosterone assessment in the rat (9426801 / 96.5%) (0014801 / 97.4%)	Reduced circulating testosterone	≥ 70 mg/kg bw/d	70 mg/kg bw/d	-	[redacted] (1998b) BASF DocID B003656
	Increased circulating LH	≥ 70 mg/kg bw/d			[redacted] (2001b) BASF DocID C019327
Leydig cell prolieration (8906201 / 97.3%)	Testicular interstitial cell proliferation	300 mg/kg bw/d	300 mg/kg bw/d	-	[redacted] (1996a) BASF DocID R014642
Plasma Testosterone measurement (0014801 / 97.4%)	Reduced circulating testosterone Increased circulating LH	≥ 70 mg/kg bw/d	70 mg/kg bw/d	6 mg/kg bw/d	[redacted] (2002c) BASF DocID C024675
Leydig cell prolieration (0014801 / 97.4%)	Testicular interstitial cell proliferation	≥ 70 mg/kg bw/d	70 mg/kg bw/d	6 mg/kg bw/d	[redacted] (2002b) BASF DocID C025273
Uterotrophic Assay (SIN0000217 / 98.1%)	No uterotrophic activity	≥ 200 mg/kg bw/d	-	400 mg/kg bw/d	[redacted] (2012a) BASF DocID 2012/1364406
Female Pubertal Assay (SIN0000217 / 98.1%)	Increased time to first oestrus	300 mg/kg bw/d	300 mg/kg bw/d	150 mg/kg bw/d	[redacted] (2012b) BASF DocID 2012/1364405
	Delayed vaginal patency				

b) Information from screening and mechanistic studies *in vitro*

There is no evidence from a number of studies *in vitro* that iprodione binds to or activates the oestrogen receptor. Kojima *et al* (2004, 2010) report a negative response for iprodione in a transactivation (reporter gene) assay using CHO cells transfected with hERα and hERβ. Vingaard *et al* (1999) similarly report a lack of activity for iprodione in the MCF7 cell proliferation assay and the Yeast Oestrogen Screen. Andersen *et al* (2002) report an absence of activity in transactivation and proliferation assays in MCF-7 cells. In a US EPA EDSP competitive binding assay (BASF DocID 2011/1294832) performed with tritiated 17β-oestradiol, iprodione at concentrations of up to 10^{-4} M was shown not to interact with oestrogen receptors isolated from rat uteri. No evidence of hERα agonism was seen in a study performed with iprodione at concentrations of up to 10^{-5} M in the stably transfected hERα-HeLa-9903 cell line (BASF DocID 2011/1294831).

There is similarly no convincing evidence from a number of studies *in vitro* that iprodione binds to or activates the androgen receptor at physiologically relevant concentrations. Fail (1994) showed an absence of binding of iprodione at concentrations of up to 10^{-4} M to androgen receptors isolated from the ventral rat prostate. In a competitive binding assay, de Groene (1999) also reports an absence of binding of iprodione to androgen receptors isolated from the T47D human cell line, at concentrations of up to 10^{-4} M. Andersen *et al* (2002) report an absence of activity in a androgen receptor transactivation assay performed in transfected CHO K1 cells. Tamura *et al* (2006) report an absence of activity for iprodione in the MDA-kb2 human cell line stably expressing an androgen-responsive reporter gene. Vingaard *et al* (2008) also report an absence of binding of iprodione in a transactivation assay using CHO K1 cells transfected with the human androgen receptor. Blystone *et al* (2009) report weak binding of iprodione to the human androgen receptor in the transiently transfected COS-1 cell line; an IC_{50} of 86 µM is reported, which is close to the limit of solubility in the assay and also approaching cytotoxic concentrations. The same authors report the inhibition of androgen-dependent gene expression in the hAR-expressing MDA-kb2 cell line. DHT-stimulated gene expression was significantly reduced at concentrations of ≥30 µM. An IC_{50} of 245.9 µM is reported for effects on gene expression, which is close to the highest (and cytotoxic) concentration of 300 µM investigated in this assay. In the absence of DHT stimulation, inhibition of gene expression was seen only at 300 µM.

Vingaard *et al* (2000) report that iprodione does not act as an inhibitor of aromatase activity in human placental microsomes. A similar lack of aromatase inhibition is reported in the US EPA EDSP study (Wilga, 2011) performed with iprodione concentrations of up to 10^{-4} M and using human aromatase, with tritiated androstenedione as the substrate. Andersen *et al* (2002) report a slight (but statistically significant) stimulation of aromatase activity in human placental microsomes by iprodione at a concentration of 50 µM. No positive control for aromatase activation was included and a similar response (weak stimulation) was seen with a number of pesticides investigated in this assay. Findings are not considered to be of biological relevance; the magnitude of change is low and is likely to be within the normal variability of the test system.

Benahmed (1995) showed that iprodione in the concentration range 1-10 µg/mL inhibits the secretion of testosterone *in vitro* by isolated porcine Leydig cells. Further investigation by the same author (Benahmed, 1996) demonstrated that iprodione inhibited Leydig cell steroidogenesis when cells were stimulated with gonadotrophin, drugs enhancing cAMP production and a cAMP analogue; however no effect was seen on gonadotrophin-stimulated cAMP production. The inhibitory effect of iprodione on steroidogenesis was not apparent in the presence of 22-*R* hydroxycholesterol. Fail (1996c) demonstrated that exposure to iprodione significantly reduced the secretion of testosterone (both with and without hCG challenge) by sections of rat testes. Comparable effects were seen in testicular sections taken from untreated rats and in testicular sections taken from rats treated with a single gavage dose of iprodione. In a steroidogenesis assay according to OECD 456 (BASF DocID 2013/1188655) the author reports the inhibition of testosterone synthesis in the H259R cell line in some replicates at iprodione concentrations of 30 and 100 µM. However in a second second study, iprodione and plant metabolites where intended to be tested for impact on steroidogenesis under the same assay conditions. Iprodione was tested up to 45µM based on cytotoxicity assessment.In contrast to the first study with iprodione, the borderline positive results could not be replicated. Therefore the study was terminated preliminary without assessment of metabolites
Therefore, the effects of iprodione on steroidogenesis in the OECD 456 study remains unclear.

Table 5.8.3-5: Summary of investigative studies *in vitro*

Study (Batch / purity)	Endpoint	Outcome	LOEC	NOEC	Reference
ER activation (unknown / 95-100%)	Oestrogen receptor transactivation in CHO cells	Negative	>10^{-5} M	10^{-5} M	Kojima *et al* (2004a) BASF DocID 2004/1036097
ER activation	Oestrogen receptor transactivation in CHO cells	Negative	>10^{-5} M	10^{-5} M	Kojima et al (2010b) BASF DocID 2010/1231472
AR activation	Androgen receptor transactivation in CHO cells	Negative			
ER activation (no info / 97.0 - 99.9%)	MCF7 cell proliferation (oestrogenicity)	Negative	>10µM	10µM	Vingaard *et al* (1999a) BASF DocID 1999/1013778
	Yeast Oestrogen Screen	Negative			
ER binding (SIN0000217 / 98.1%)	Rat uterus oestrogen receptor binding	Negative	>10^{-4} M	10^{-4} M	Willoughby (2011a) BASF DocID 2011/1294832
ER activation (SIN0000217 / 98.1%)	Oestrogen receptor agonism in transfected HeLa cells	Negative	>10^{-5} M	10^{-5} M	Willoughby (2011b) BASF DocID 2011/1294831
ER activation (no info / 96.8 - 99.9%)	Oestrogen activity (E-Screen)	Negative	>10^{-5} M	10^{-5} M	Boehmler (2004a) BASF DocID 2013/1347912
AR binding (8906201 / 97.3%)	Rat prostate androgen receptor binding	Negative	>10^{-4} M	10^{-4} M	Fail (1994a) BASF DocID C024395
AR binding (TV3015C / 98.1%)	T47D human cell line androgen receptor binding	Negative	>10^{-4} M	10^{-4} M	de Groene (1999a) BASF DocID R014678
AR activation (no info / no info)	MDA-kb2 human cell line androgen receptor activation	Negative	unknown	unknown	Tamura *et al* (2006a) BASF DocID 2006/1050814
AR activation (no info / no info)	Human androgen receptor activation in transfected CHO K1 cells	Negative	>30µM	30µM	Vingaard *et al* (2008a) BASF DocID 2008/1101756
AR activation (MO07526JO / 97%)	Human androgen receptor binding in transfected COS-1 cells	Weak binding at high conc.	IC_{50} 86µM	-	Blystone *et al* (2009a) BASF DocID 2008/1101757
	Androgen-dependent gene expression in the MDA-kb2 cell line	Inhibition at very high conc. (questionable)	IC_{50} 246µM	-	

Study (Batch / purity)	Endpoint	Outcome	LOEC	NOEC	Reference
CYP19 Activation (no info / 97.0 - 99.9%)	Aromatase inhibition in human placental microsomes	Negative	>50µM	50µM	Vingaard *et al* (1999b) BASF DocID 1999/1013777
CYP19 Activation (SIN0000217 / 98.1%)	Human aromatase inhibition	Negative	$>10^{-4}$ M	10^{-4} M	Wilga (2011a) BASF DocID 2011/1294833
Estrogenicity	ER transactivation assay	negative	$>10^{-5}$ M	10^{-5} M	Andersen (2002a)
	MCF-7 cell proliferation (oestrogenicity)	negative	$>10^{-5}$ M	10^{-5} M	BASF DocID 2001/1031823
Androgenicity	AR transactivation assay	negative	$>10^{-5}$ M	10^{-5} M	
CYP19 activity (no info / no info)	Aromatase inhibition in human placental microsomes	weak stimulation (questionable)	50µM	<50µM	
Testosterone secretion (TV3015C / 98.1%)	Porcine Leydig cells Testosterone secretion	inhibition	1µg/mL	<1µg/mL	Benahmed (1995a) BASF DocID R014672
Testosterone secretion (TV3015C / 98.1%)	Porcine Leydig cells Testosterone secretion	inhibition	10µg/mL	<10µg/mL	Benahmed (1996a) BASF DocID C029154
Testosterone secretion (8906201 / 97.3%)	Rat testes sections Testosterone secretion	inhibition	1µg/mL	<1µg/mL	Fail (1996c) BASF DocID C024292
Steroidogenesis (MD2085 / 99.8%)	Steroidogenesis in human H259R cell line	Borderline positive	30µM	10µM	R. Hernandez (2013a) BASF DocID 2013/1188655

5.8.3.5 Mode of action of iprodione

Findings in the standard repeated dose toxicity studies performed with iprodione in all species investigated indicate effects on tissues and organs of the endocrine system. The most sensitive target is shown to be the adrenal in all species investigated.

The repeated dose toxicity studies show effects of treatment that are seen both in tissues and organs responsive to steroid hormones and also in tissues and organs involved in steroid hormone synthesis. Although findings are noted in reproductive tract tissues in both sexes, studies of reproductive toxicity do not indicate any effect of iprodione on fertility, reproductive function or capacity, including sperm parameters. The most recent reproductive toxicity study (BASF DocID 2013/1251918) identified delayed preputial separation in male offspring as a sensitive indicator. A NOAEL of 300 ppm (32 mg/kg bw/d) was determined for this study. Findings are consistent with the delay in preputial separation reported by Blystone *et al* (2007), which reported a NOEL of 50 mg/kg bw/d. While these effects suggest an anti-androgenic mechanism for iprodione, findings in other studies *in vivo* (notably effects on non-androgen-sensitive tissues such as the adrenals) are not consistent with this and indicate a more general effect on steroidogenesis. It is also notable that the overall NOEL for adrenal pathology of 31 mg/kg bw/d (BASF DocID R014676) is highly consistent with the NOELs from the studies of Blystone *et al* (2007) and the latest generation study in rats (BASF DocID 2013/1251918).

Studies *in vitro* demonstrate the inhibition of testicular testosterone synthesis and/or release by porcine testicular interstitial cells and in sections of rat testes incubated with iprodione. Studies *in vivo* also show a rapid and transient reduction in the levels of circulating testosterone following iprodione treatment; however a potentially concomitant reduction in the testosterone precursors androstenedione and 17α-hydroxyprogesterone would demonstrates a more general inhibition of steroidogenesis. The iprodione-mediated inhibition of testosterone synthesis *in vitro* was seen in cells stimulated with cholera toxin, forskolin (which enhances cAMP production) or the cAMP analogue 8-bromo-cAMP. iprodione had no effect on gonadotrophin-stimulated cAMP production and inhibitory effect of iprodione on testosterone synthesis was not apparent in the presence of 22-*R* hydroxycholesterol, a substrate which does not require active transport into the mitochondria. The results of these investigative studies therefore suggest that the effect of iprodione on testosterone synthesis is due to prevention of the active transport of cholesterol from the cytosol into the mitochondria. Iprodione thus prevents the conversion of cholesterol to pregnenolone by cytochrome P450scc, which is the initial stage in the synthesis of all steroid hormones in the adrenals, testes and ovaries. The primary site of iprodione activity is therefore likely to be the steroidogenic acute regulatory (StAR) protein, the rate-limiting step in steroid hormone synthesis.

A non-specific effect of iprodione on steroidogenesis, as indicated by the extensive mechanistic data, potentially results in lower levels of circulating androgens and, consequently, will result in effects on a number of target tissues. Findings in repeated dose studies performed with iprodione are therefore consistent with the proposed mechanism. Effects are seen in various studies on the male and female reproductive tracts and on the adrenal gland. Effects on the adrenal are characterised by enlargement, increased weight, vacuolisation and/or hypertrophy of cells of the adrenal cortex. Findings are apparent in all areas of the adrenal cortex (i.e. the *zona glomerulosa*, *zona fasciculata* and *zona reticularis*), indicating a general effect on steroidogenesis. Adrenal findings may reflect the accumulation of cholesterol within cells of the adrenal cortex as a consequence of the blockade of cholesterol transport. The dataset shows effects likely to be a consequence of the general inhibition of steroidogenesis, consequent reductions in androgen levels and homeostatic responses. Endpoints consistent with a reduction in circulating androgen levels are among the most sensitive, with an overall NOEL very similar to that demonstrated for adrenal pathology.

The findings of the investigative studies are also consistent with observations of interstitial cell proliferation in the repeated dose toxicity and carcinogenicity studies and are consistent with the proposed threshold, non-genotoxic mode of action for interstitial cell carcinogenicity in the male rat. While the small increase in the incidence of benign ovarian luteoma seen in female mice at the highest dose level of 4000 ppm (600 mg/kg bw/d) in the mouse carcinogenicity study (BASF DocID R014586) is also consistent with the proposed mode of action, subsequent analysis (BASD DocID B003580), concluded that the incidence was not statistically significant and was within the laboratory's historical control range. An increase in ovarian cell hyperplasia and luteinisation was considered to be related to treatment. The inhibition of steroidogenesis in ovarian thecal cells will result in a reduction in androstenedione supplied to the adjacent ovarian granulosa cells. A consequent reduction of oestradiol release from the granulosa cells will result in a homeostatic increase in the secretion of GnRH by the hypothalamus and FSH and LH by the pituitary and stimulation of the ovarian thecal cells.

5.8.3.6 Conclusion

Mechanistic data for iprodione indicate a general inhibition of steroidogenesis. Relevant endocrine-related findings from the repeated dose studies identify adrenal histopathology as the most sensitive endpoint. The most recent reproductive toxicity study includes assessment of endocrine-sensitive endpoints and identifies delayed sexual maturation of male offspring (as well as adrenal vacuolation) as the most sensitive endpoints. It is notable that, across the repeated dose, reproductive and mechanistic studies performed with iprodione, (no) effect levels are similar. Studies consistently identify NOEL values at dose levels of approximately 30 mg/kg bw/d or greater. Consistent NOEL values are those of 31 mg/kg bw/d from the 90-day study (BASF DocID R014676), a NOAEL of 32 mg/kg bw/d from the new multi-generation study (BASF DocID 2013/1251918) and a NOEL of 50 mg/kg bw/d from the mechanistic study of Blystone *et al* (2007). Lower NOEL values (6 mg/kg bw/d) are reported (BASF DocID C024675), however the LOELs for these studies (70 mg/kg bw/d) are consistent with those from other studies. Studies in which NOELs were not identified (BASF DocID B003656, BASF DocID C019327), also report LOELs of 70 mg/kg bw/d; effects were not investigated at lower dose levels. The dataset in its entirety is therefore consistent, and identifies a threshold for the endocrine related effects of iprodione (based on the analysis of a number of relevant endpoints), and an overall NOEL of approximately 30 mg/kg bw/d and a LOEL of approximately 70 mg/kg bw/d.

Peer reviewed literature

Report:	CA 5.8.3/1 Blystone C.R. et al., 2007a Iprodione delays male rat pubertal development, reduces serum testosterone levels, and decreases ex vivo testicular testosterone production 2008/1101757
Guidelines:	none
GLP:	no

Executive Summary of the Literature

The authors assessed male rat pubertal development, testosterone production within the testis after treatment with iprodione and competitive androgen receptor binding. For this purpose, Sprague-Dawley weanling rats were dosed by gavage with 0, 50, 100, or 200 mg/kg/day of iprodione (purity 97%, Lot#: MO07526JO) from post-natal day (PND) 23 to 51/52. The onset of puberty (progression of preputial separation (PPS)) was measured starting on PND 37. Organ weights, serum hormones, and ex vivo testis steroid hormone production under stimulated (+human chorionic gonadotropin (hCG)) and unstimulated (-hCG) conditions were measured at necropsy.

iprodione delayed PPS at 100 and 200 mg/kg/day and decreased androgen sensitive seminal vesicle and epididymides weights at 200 mg/kg/day. Furthermore, iprodione increased adrenal and liver weights at 200 mg/kg/day, presumably by different mechanism(s) of action.

BASF assessment: No information is given on the laboratory historical control data on initiation and completion day of preputial separation as well as on organ weight at day of necropsy. The most relevant and GLP-conform study is the 2-generation study by Schneider S., et al., 2013, the information given in this study is regarded as supportive.

Serum testosterone levels were decreased ($p < 0.0001$) by 73%, 83%, and 89% of the control value at 50, 100, and 200 mg/kg/day doses, respectively along with serum 17 alpha-hydroxyprogesterone and androstenedione whereas serum LH was unaffected. iprodione did not significantly ($p = 0.0980$) alter (decrease or increase) serum progesterone.

BASF assessment: Animals were necropsied within 1.25–3.50 h after final dosing and trunk blood was collected for serum hormone measurements. In other mechanistic studies at similar dose-ranges the peak of plasma testosterone level reduction occurs between 0.5 – 4h and return to normal within 24 hours (et al., 1998b, 2001b; ., 2002c). Therefore, the sensitive period of plasma testosterone level was assayed in this study. The authors report that LH levels were unaffected. However, the decrease in testosterone levels is followed by an increase in LH levels with a peak beyond 4h after last treatment (., 1998b, 2001b; ., 2002c). Therefore the sensitive time point for LH measurement was missed in the current study and the interpretation by the authors that "IPRO did not increase serum LH in the current study (…) even though it reduced serum testosterone" and that the proposed mode of action, that is chronic exposure to high levels of LH resulting in Leydig cell tumor formation, cannot be supported is not valid. In addition, no information is given on the reversibility of plasma testosterone levels nor was an assessment performed on testis cell proliferation. Other hormone measurements than testosterone and progesterone (unaffected) were not valid as the authors state that "the results of the serum steroid measurements other than testosterone and progesterone were problematic, since they were at such low levels in these young males that many of the values were below the levels of detection". Ex vivo testis production of testosterone and progesterone was decreased. In summary, the results on plasma hormone level measurements are supportive of the results reported by 1998b; 2001b and ., 2002c; 2002b.

The authors report the failure of iprodione to elicit an AR antagonism in vitro, providing evidence that iprodione differs from the dicarboximides procymidone and vinclozolin in that the effects on male rat pubertal development result from an inhibition of steroidogenesis and not AR antagonism.

Classification of study: Supplementary information

Report: CA 5.8.3/2
Blystone C.R. et al., 2009a
Cumulative and antagonistic effects of a mixture of the antiandrogens Vinclozolin and Iprodione in the pubertal male rat
2009/1130622
Guidelines: none
GLP: no

Executive Summary of the Literature

The authors report that iprodione (purity 97%, Lot#: MO 07526JO) binds to the human androgen receptor (IC_{50} = 86.0µM), reduces androgen-dependent gene expression, and reduces androgen-sensitive tissue weights in castrated male rats (Hershberger assay).

BASF assessment: The weak binding of iprodione to the human androgen receptor in the transiently transfected COS-1 cell line is close to the limit of solubility in the assay and also approaching cytotoxic concentrations. Many other studies (see summary on *in vitro* studies and references listed in this chapter on androgen receptor binding/activation as well as de Groene E.M. et al., 1999b Chapter 5.8.2) report negative binding/activation to the androgen receptor by iprodione in the cell systems and conditions used in the assessments. The same authors report the inhibition of androgen-dependent gene expression in the hAR-expressing MDA-kb2 cell line. DHT-stimulated gene expression was significantly reduced at concentrations of ≥30 µM. An IC_{50} of 245.9 µM is reported for effects on gene expression, which is close to the highest (and cytotoxic) concentration of 300 µM investigated in this assay. In the absence of DHT stimulation, inhibition of gene expression was seen only at 300 µM. Reduced androgen sensitive tissue weights were reported at the highest dose tested (200mg/kg bw/d; reduced ventral prostate weight, reduced LABC). At lower iprodione concentrations the only statistical significantly anti-androgen sensitive endpoint changed was LABC weight (reduced). However, as this was the only anti-androgen related finding at these dose levels in the Hershberger assay and no historical control data were reported to relate the change to normal variation of laboratory control animals, the biological relevance of endocrine related findings below 200mg/kg bw/d are of questionable relevance.

Classification of study: Supplementary information

Report: CA 5.8.3/3
Kojima H. et al., 2004a
Screening for estrogen and androgen receptor activities in 200 pesticides by in vitro reporter gene assays using Chinese hamster ovary cells
2004/1036097
Guidelines: none
GLP: no

Executive Summary of the Literature

200 pesticides, including iprodione (purity >95%), were tested for their agonistic and antagonistic activity towards the human estrogen receptor (hER) subtypes α and β as well as the human androgen receptor (hAR) in transactivation assays using chinese hamster ovary (CHO) cells.
In this Luciferase reporter gene assay the pesticides were evaluated for their agonistic activity based on relative activity, expressed as that concentration that showed 20 % of the activity of 0.1 nM estradiol (E2), 1 nM E2 or 1nM dihydrotestosterone (DHT) for hER, hER or hAR, respectively. The antagonistic activity was evaluated based on the relative inhibitory activity, expressed as that concentration that showed 20 % inhibition of the activity induced by 0.01 nM E2, 0.1 nM E2 or 0.1 nM DHT for hER, hER or hAR, respectively. The pesticides were all tested up to a concentration of 10 μM to avoid cell toxicity.

iprodione was negative in the hER-alpha, hER-beta and hAR assays.

Classification of study: Supplementary information

Report: CA 5.8.3/4
Kojima H. et al., 2010a
Comparative study of human and mouse pregnane X receptor agonistic activity in 200 pesticides using in vitro reporter gene assays
2013/1347909
Guidelines: none
GLP: no

Executive Summary of the Literature

A variety of environmental chemicals were tested in vitro for their agonistic activity to the human and mouse pregnane X receptor (hPXR and mPXR). A reporter gene assay based on the simian kidney cell line COS-7 was used as test system. iprodione was not among the compounds activating human- or mousePXR.

Classification of study: Supplementary information

Report: CA 5.8.3/5
Vinggaard A.M. et al., 1999a
Screening of selected pesticides for oestrogen receptor activation in vitro
1999/1013778
Guidelines: none
GLP: no

Executive Summary of the Literature

Twenty pesticides were tested for their ability to activate the oestrogen receptor in vitro using an MCF7 cell proliferation assay and a Yeast Oestrogen Screen. iprodione (purity >97%) did not affect MCF-7 cell proliferation nor an oestrogenic response in the Yeast Oestrogen Screen at 10µM.

Classification of study: Supplementary information

Report: CA 5.8.3/6
Boehmler G.,Borowski U., 2004a
Nachweis estrogener Wirksamkeit mit einem biologischen Testsystem - Teil 2: Untersuchung lebensmittelrelevanter Einzelsubstanzen
2013/1347912
Guidelines: none
GLP: no

Executive Summary of the Literature

17 dietary exposure relevant substances were tested in the E-Screen assay for estrogen mediated activity. iprodione (purity >96.8%) was negative tested at concentrations ranging from 10^{-5} to 10^{-9}.

Classification of study: Supplementary information

Report: CA 5.8.3/7
Tamura H. et al., 2006a
Structural basis for androgen receptor agonists and antagonists: Interaction of SPEED 98-listed chemicals and related compounds with the androgen receptor based on an in vitro reporter gene assay and 3D-QSAR
2006/1050814
Guidelines: none
GLP: no

Executive Summary of the Literature

The androgen receptor (AR) activity of listed chemicals, so called SPEED 98, by the Ministry of the Environment, Japan, and structurally related chemicals was characterized using MDA-kb2 human breast cancer cells stably expressing an androgenresponsive luciferase reporter gene, MMTV-luc. iprodione (unknown purity) was negative in the conditions of this assay.

Classification of study: Supplementary information

Report: CA 5.8.3/8
Vinggaard A.M. et al., 2007a
Screening of 397 chemicals and development of a quantitative structure-activity relationship model for androgen receptor antagonism
2008/1101756
Guidelines: none
GLP: no

Executive Summary of the Literature

The authors screened 397 chemicals for human androgen receptor (AR) antagonism by a sensitive reporter gene assay in chinese hamster ovary cells. iprodione (unknown purity) was negative up to the highest dose tested (30µM).

Classification of study: Supplementary information

Report: CA 5.8.3/9
Vinggaard A.M. et al., 1999b
Screening of selected pesticides for inhibition of CYP19 aromatase activity in vitro
1999/1013777
Guidelines: none
GLP: no

Executive Summary of the Literature

Twenty-two pesticides were tested for their ability to affect CYP19 aromatase activity in human placental microsomes using the classical $[^3H]_2O$ method. iprodione (purity >97%) does not act as an inhibitor of aromatase activity in human placental microsomes at doses up to 50µM.

Classification of study: Supplementary information

Report: CA 5.8.3/10
Andersen H.R. et al., 2001a
Effects of currently used pesticides in assays for estrogenicity, androgenicity, and aromatase activity in vitro
2001/1031823
Guidelines: none
GLP: no

Executive Summary of the Literature

Twenty-four pesticides were tested for interactions with the estrogen receptor (ER) and the androgen receptor (AR) in transactivation assays. Estrogen-like effects on MCF-7 cell proliferation and effects on CYP19 aromatase activity in human placental microsomes were also investigated. The authors report an absence of activity in transactivation and proliferation assays in MCF-7 cells and an absence of activity in an androgen receptor transactivation assay performed in transfected CHO K1 cells with iprodione (unknown purity). The authors also report a slight (but statistically significant) stimulation of aromatase activity in human placental microsomes by iprodione at a concentration of 50 µM.

BASF assessment: No positive control for aromatase activation was included and a similar response (weak stimulation) was seen with a number of pesticides investigated in this assay. Findings are not considered to be of biological relevance; the magnitude of change is low and is likely to be within the normal variability of the test system which has not been reported in this study.

Classification of study: Supplementary information

Report: CA 5.8.3/11
Kojima H. et al., 2010b
Endocrine-disrupting potential of pesticides via nuclear receptors and aryl hydrocarbon receptor
2010/1231472
Guidelines: none
GLP: no

Executive Summary of the Literature

In this review, the authors present transactivation assay-based screening results for 200 pesticides against nuclear receptors and AhR. iprodione (unknown purity) did not transactivate estrogen receptors (ERs), androgen receptor (AR), thyroid hormone receptors (TRs), pregnane X receptor (PXR), peroxisorne proliferator-activated receptors (PPARs), and AhR under the conditions of these assays.

Classification of study: Supplementary information

Report: CA 5.8.3/12
Ghisari M.,Bonefeld-Jorgensen E.C., 2005a
Impact of environmental chemicals on the thyroid hormone function in pituitary rat GH3 cells
2005/1043040
Guidelines: none
GLP: no

Executive Summary of the Literature

The authors investigated the thyroid hormone (TH) disrupting activity of different classes of endocrine disrupting compounds including plasticizers (bisphenol A, bisphenol A dimethacrylate), alkylphenols (4-n-nonylphenol, 4-octylphenol), pesticides (prochloraz, iprodion, chlorpyrifos), PCB metabolites (OH-PCB 106, OH-PCB 121, OH-PCB 69) and brominated flame-retardants (tetrabromobisphenol A). The ED potential of a chemical was determined by its effect on the cell proliferation of TH-dependent rat pituitary GH3 cell line. All tested chemicals significantly interfered with the cell proliferation alone or upon co-treatment with T3. The growth of GH3 cells was stimulated by all tested chemicals, but 4-n-nonylphenol, 4-octylphenol, prochloraz and iprodion (purity >95%) elicited an inhibitory effect on cell growth.

BASF assessment: The test was performed on a non-validatd method. Cytotoxicity analysis results performed with iprodione were not reported. High variability in the measurements was evident across all compounds and all measurements. No dose-response was seen on cell proliferation in vitro. The U-shaped dose response was based on the highest dose tested, displaying decreased cell proliferation which, at the concentration tested, may have been associated to cell toxicity rather than a compound specific effect. No compound related effects in the standard repeated dose toxicity studies were seen on thyroid in rats, mice, dogs.

Classification of study: non-reliable

Report:	CA 5.8.3/13 Takeuchi S., 2006a In vitro screening of 200 pesticides for agonistic activity via mouse peroxisome proliferator-activated receptor (PPAR)Alpha and PPARgamma and quantitative analysis of in vivo induction pathway 2006/1050813
Guidelines:	none
GLP:	no

Executive Summary of the Literature

In the present study, the authros characterized mouse PPAR-alpha and PPAR-gamma agonistic activities of 200 by in vitro reporter gene assays using CV-1 monkey kidney cells. iprodione (purity >95%) did not activate the PPAR-alpha/gamma in vitro

Classification of study: Supplementary information

Report: CA 5.8.3/14
Takeuchi S. et al., 2008a
In vitro screening for aryl hydrocarbon receptor agonistic activity in 200 pesticides using a highly sensitive reporter cell line, DR-EcoScreen cells, and in vivo mouse liver cytochrome P450-1A induction by Propanil, Diuron and Linuron
2013/1347911
Guidelines: none
GLP: no

Executive Summary of the Literature

A variety of environmental chemicals were tested in vitro for their agonistic activity towards the aryl hydrocarbon receptor (AHR) in DR-Eco Screen cells. In addition propanil, diuron and linuron were tested in vivo in C57BL/6 mice.
iprodione (purity >95%) did not induce AHR mediated signalling.

Classification of study: Supplementary information

Report: CA 5.8.3/15
Shah I. et al., 2011a
Using nuclear receptor activity to stratify hepatocarcinogens
2011/1295091
Guidelines: none
GLP: no

Executive Summary of the Literature

As part of the ToxCast program iprodione was tested for activity on human constitutive androstane receptors (CAR/NR1I3), pregnane X receptor (PXR/NR1I2), aryl hydrocarbon receptor (AhR), peroxisome proliferator-activated receptors (PPAR/NR1C), liver X receptors (LXR/NR1H), retinoic X receptors (RXR/NR2B) and steroid receptors (SR/NR3) in vitro. iprodione was positive in activation assays for ATG_Ahr_CIS, ATG_PPARg_TRANS, ATG_PXR_TRANS, ATG_PXRE_CIS, CLZD_CYP1A1_24, CLZD_CYP1A2_24, CLZD_CYP2B6_24, CLZD_CYP3A4_24, NVS_NR_hPXR
It is interesting to note, that iprodione was not associated with the activation of other assays targeting estrogen receptor and/or androgen receptor.

Classification of study: Supplementary information

Report: CA 5.8.3/16
Sipes N.S. et al., 2013a
Profiling 976 toxcast chemicals across 331 enzymatic and receptor signaling assays
2013/1371960
Guidelines: none
GLP: no

BASF response to in vivo studies – The presented in vivo studies present some evidence of iprodione targeting androgen dependent activity most likely by affecting general steroidobiosynthesis but not by interacting with the androgen receptor.

BASF response to in vitro studies – In vitro studies performed with iprodione were conducted in highly artificial cell systems and displayed no binding or transactivation of androgen or estrogen receptors. In addition, iprodione most likely does not affect aromatase. The assays applied are in general lacking any form of metabolic competence which is especially critical in a setting where the substance is rapidly and nearly completely metabolized as is the case for iprodione.
In a recent evaluation of the predictivity of the high-throughput in vitro screening battery used in ToxCast, Thomas et al (Toxicological Sciences; 128(2), 398-417 (2012)) tested 84 statistical classification models (JMP Genomics software 5.0) with chemical descriptors (QSAR analysis) or in vitro assays as variables. After multiple iterations and cross validations, they identified balanced accuracy scores to be < 0.55 (or 55% predictivity) for 56 of the 60 endpoints. There was little to no predictive advantages of the cell based assays in comparison to QSAR tools, which are generally deemed to have low predictivity for complex endpoints (<70% accuracy, evaluation of multiple endpoints by EPA and EFSA).

This indicates that in vitro studies only provide an initial step in the evaluation of the toxicological properties of a compound.

CA 5.9 Medical Data

A search in the databases listed below - restricted to "pps=human" and "ct d human" - has been performed on March 10, 2014 via DIMDI-host for the following terms:
CR=36734-19-7 or ct=iprodione or ft= iprodion?

Medline 66 (NLM)
Medline alert (NLM)
Embase 74 (Elsevier)
Embase alert (Elsevier)
Cochrane Library-Central
Biosys (Thomson Reuters)
gms (German Medical Science)
IPA International Pharmaceutical Abstracts (Thomson Reuters)
Deutsches Aerzteblatt (Aerzteverlag)

Cross check via Internet available databases:
CHEMID (http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp)
PubMed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi)
Toxnet (http://toxnet.nlm.nih.gov/)

These searches revealed no relevant documents.

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 iprodione. Thus, the medical monitoring program 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. Except of one case of eye irritation no adverse health effects suspected to be related to iprodione exposure have been observed.

In France, measurement of blood parameters carried out between 1977 and 1988 on employees involved in the synthesis and packaging of iprodione are summarized in a letter from Dr Bioche (1988).
From a medical point of view, the only problems observed have been dry hands without signs of eczema in two workers involved in packaging. All signs disappeared with a change of soap.
Other medical problems arose in the chemical synthesis stations where contact was mainly with intermediate reagents.
Results from analyses conducted in France, during 1989 (Bioche, 1990) did not show any adverse effect among workers involved in the final stages of iprodione production.
Results confirmed for the period 1990-1993 (Kaltwasser, C. 1993)
No adverse effects were observed during the wettable powder process (Nougaret, D.,1993; Urtizberea, M. 1993b).
From 1993 until April 2006 furthermore no adverse effects or clinical signs related to iprodione production were observed (Barré 2006).

CA 5.9.2 Data collected on humans

One case of slight eye irritation has been registered in the BASF-internal clinical incident log in employees exposed to iprodione

CA 5.9.3 Direct observations

Same cases of skin irritation have been observed in Iprodione-exposed workers. In operators spraying Iprodione -containing compounds nausea and breathing difficulties, have been reported. However, it is unclear whether pure Iprodione stored only for a short time also is a potential sensitizer or whether the allergic reactions are due to impurities/decomposition products.

CA 5.9.4 Epidemiological studies

Neither data on exposure of the general public nor epidemiologic studies are available for BASF SE, nor is BASF SE aware on any epidemiologic studies performed by third parties.

CA 5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

3,5-Dichloroaniline is a biomarker for Iprodione and can be detected in human urine by chromatography.
There are no specific signs of intoxication or no known clinical tests for poisoning in humans.

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

BAS 610F

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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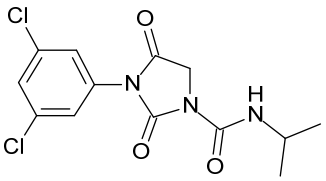
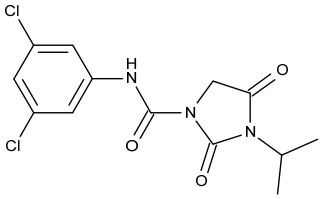
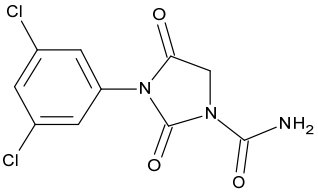
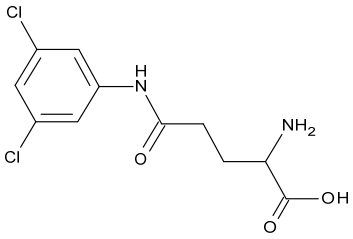
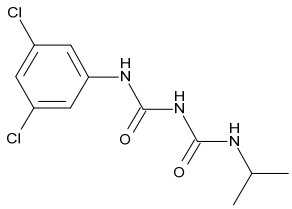
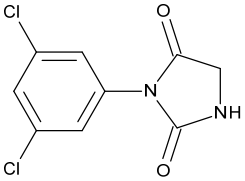
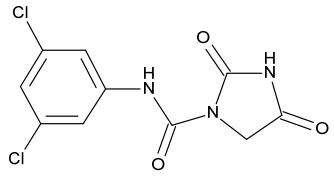
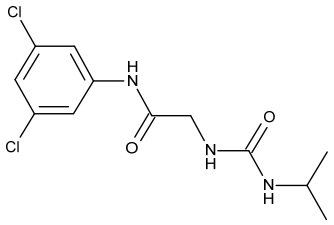
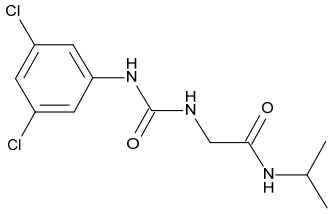
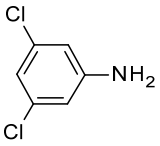
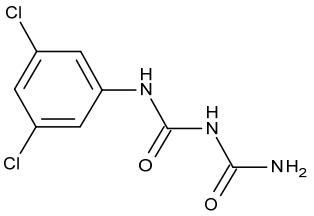
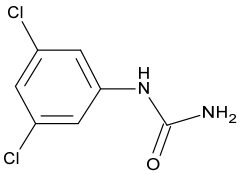
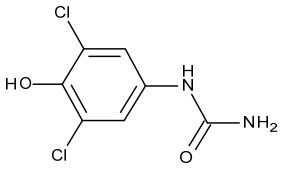
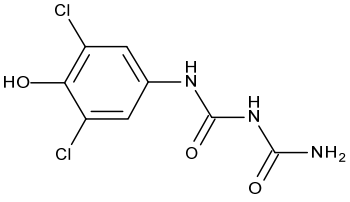
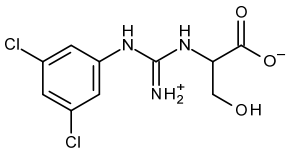
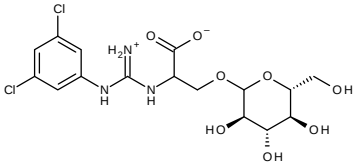
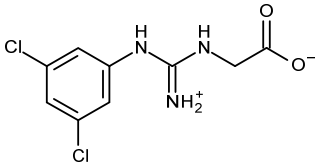
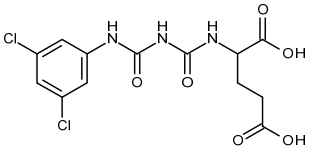
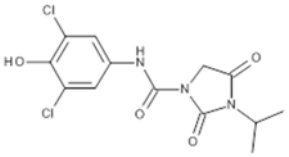
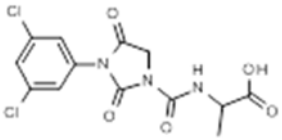
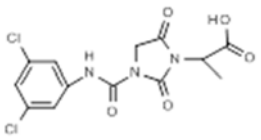
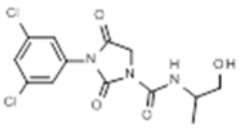
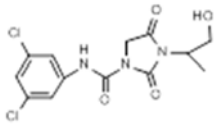
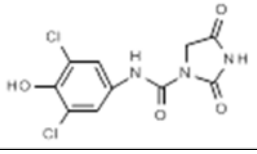
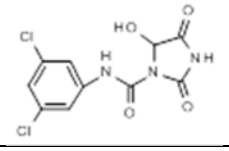
CA 6 RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED AND PLANT METABOLISM

A concordance list of structures and designations of reference compounds used during Consumer Safety studies is given below.

Compound designation [1)]	Reference code (Reg. No.)	Formula	Molecular weight (g/mol)	Structure
Iprodione RP 26019 M610F000	101169	$C_{13}H_{13}Cl_2N_3O_3$	330.17	
RP 30228 M610F001	5079647	$C_{13}H_{13}Cl_2N_3O_3$	330.17	
RP 32490	5079628	$C_{10}H_7Cl_2N_3O_3$	288.09	
M610F007	5916256 (L-Form)	$C_{11}H_{12}Cl_2N_2O_3$	291.13	
RP 36221 M610F002	5079618	$C_{11}H_{13}Cl_2N_3O_2$	290.15	
RP 25040 M610F004	207099	$C_9H_6Cl_2N_2O_2$	245.1	

Compound designation [1)]	Reference code (Reg. No.)	Formula	Molecular weight (g/mol)	Structure
RP 36112 M610F003	5079623	$C_{10}H_7Cl_2N_3O_3$	288.1	
RP 37176	5079612	$C_{12}H_{15}Cl_2N_3O_2$	304.18	
RP 36233	5079632	$C_{12}H_{15}Cl_2N_3O_2$	304.18	
RP 32596, 3,5-DCA M610F012	85831	$C_6H_5Cl_2N$	162.02	
RP 36115 M610F005	5079624	$C_8H_7Cl_2N_3O_2$	248.07	
RP 44247 M610F006 LS720942	89517	C7 H6 Cl2 N2 O	205.0	
DCHPU	5932706	C7H6Cl2N2O2	221.04	
RP 36114	5079627	$C_8H_7Cl_2N_3O_3$	264.07	

Compound designation [1)]	Reference code (Reg. No.)	Formula	Molecular weight (g/mol)	Structure
M610F008		$C_{10}H_{11}Cl_2N_3O_3$	292.1	
M610F009		$C_{16}H_{21}Cl_2N_3O_8$	454.3	
M610F010		$C_9H_9Cl_2N_3O_2$	262.1	
M610F011		$C_{13}H_{13}Cl_2N_3O_6$	378.2	
RP 36119	5079629	$C_{13}H_{13}Cl_2N_3O_4$	346.2	
RP 36118	5079631	$C_{13}H_{11}Cl_2N_3O_5$	360.2	
RP 36116	5079630	$C_{13}H_{11}Cl_2N_3O_5$	360.2	
RP 36117		$C_{13}H_{13}Cl_2N_3O_4$	346.2	
RP 36111		$C_{13}H_{13}Cl_2N_3O_4$	346.2	
RP 36110		$C_{10}H_7Cl_2N_3O_4$	305.1	

Compound designation [1)]	Reference code (Reg. No.)	Formula	Molecular weight (g/mol)	Structure
RP 36113		$C_{10}H_7Cl_2N_3O_4$	305.1	

1) For RP codes, different formats are in use:
"RP xxxxx" or or "RPxxxxx" or "RP0xxxxx" or " RP-xxxxx"
independent from these format differences, the last 5 digits "xxxxx" are unique for every compound

CA 6.1 Storage stability of residues

Freezer storage stability of residues in plant matrices to support residue trials is demonstrated in the studies provided below.

Report:	CA 6.1/1 Plaisance R.S., 1994a Storage stability of Iprodione (RP-26019), its isomer (RP 30228), and its metabolite (RP-32490) in various raw agricultural commodities and processing fractions C022404
Guidelines:	EPA 171-4(e)
GLP:	Yes (certified by United States Environmental Protection Agency)

Report:	CA 6.1/2 Gillings O., 1995a A study in support of the report, Storage stability of Iprodione (RP-26019), its isomer (RP-30228), and its metabolite (RP-32490) in various raw agricultural commodities and processing fractions C022407
Guidelines:	EPA 171-4(e)
GLP:	Yes (certified by United States Environmental Protection Agency)

Executive Summary

A freezer storage stability study was performed investigating several plant samples spiked with iprodione (RP-26019), its isomer (RP-30228) and its metabolite (RP-32490) at 5.0 mg/kg. The study was performed over a period of 12 months. In an additional study, one further time point between 24 and 34 months was investigated for selected plant material. The samples were analyzed for iprodione, its isomer and its metabolite with HPLC-UV.
The data indicate that residues of iprodione, its isomer and its metabolite are stable when stored at -10°C for up to 12 months. Residues of all three compounds were not stable in peanut soapstock because of the high pH of this substrate. There were no general trends indicating that any of the residues were declining with time. The results varied within what would be expected to be normal analytical variability with residue analyses over several months. The results from the extended storage stability study lengthen that storage stability period, because the data obtained clearly indicate that iprodione, its isomer and its metabolite are stable for 24 to 34 months when stored under frozen conditions. Any variations of results fell within normal expectations for analyses of residue substrates.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Iprodione (RP-26019); RP-30228; RP-32490
Description:
Lot/Batch #: EA2002SD8; EA2025RF1; EA2026RF1
Purity: 99.9% (99.7%); 98.1% (99.6%); >95.0% (99.7%)
CAS#: 36734-19-7; 63637-89-8; 79076-80-5
Spiking levels: 5.0 mg/kg

2. Test Commodity:
Crop: Almonds (hulls, nutmeat), apples, blueberries, broccoli, oilseed rape (presscake, seed, crude and refined oil), carrot, maize, cotton, cucumber, garlic (dry bulb), ginseng (root), grapes (fruit, wet and dried pomace, raisins, raisins waste, juice), lettuce, onion, peaches, peanuts (hay, hulls, nutmeat, vines, meal, crude and refined oil, soapstock), peppers, potatoes (chips, granules), rice (bran, grain, hulls, polished, straw), strawberry, tobacco and tomato
Sample size: 10 g (aliquots)

B. STUDY DESIGN AND METHODS

1. Test procedure

The stability of iprodione (RP-26019), its isomer (RP-30228) and its metabolite (RP-32490) was investigated under deep frozen conditions (-10°C) over a time period of 12 months in almonds (hulls, nutmeat), apples, blueberries, broccoli, oilseed rape (presscake, seed, crude and refined oil), carrot, maize, cotton, cucumber, garlic, ginseng (root), grapes (fruit, wet and dried pomace, raisins, raisins waste, juice), lettuce, onion, peaches, peanuts (hay, hulls, nutmeat, vines, meal, crude and refined oil, soapstock), peppers, potatoes (chips, granules), rice (bran, grain, hulls, polished, straw), strawberry, tobacco and tomato.
The samples were spiked with the test items at a concentration level of 5.0 mg/kg. The spiked samples were stored frozen and analyzed after different intervals (0, 3, 6, 9 and 12 months).
In an additional study, extended storage periods were tested for selected plant material: almonds (hulls, nutmeat), carrot, maize, cotton, lettuce and tomato. One further time point between 24 and 34 months was investigated per matrix.

2. Description of analytical procedures

The method used for the analysis of all substrates was, "Method of Determination for Iprodione (RP-26019), its Isomer (RP-30228), and its Metabolite (RP-32490) in Various Raw Agricultural Commodities and Processing Fractions". The limit of quantitation for this study was 0.25 mg/kg.
A representative portion of the crop sample was extracted with acetonitrile and water receiving a pH adjustment with hydrochloric acid. Depending on the substrate involved, a hexane:acetonitrile:water partition, Florisil™ column chromatography, and gel permeation chromatography (GPC) were performed. After the aqueous and organic extracts were concentrated, the sample was analyzed for iprodione, its isomer and its metabolite with reverse phase high performance liquid chromatography and UV detection at 200 or 210 nm. Individual residues were reported as either iprodione (RP-26019), its isomer (RP-30228) or its metabolite (RP-32490).

Method recoveries were analyzed with each set of stored samples. A total of 208 method spikes were run. Each method spike contained iprodione, its isomer, and its metabolite resulting in 524 recovery values. The overall range of individual recovery values was 41 to 139% with a mean of 93 ± 12%. Only four percent of the individual compound recovery values (26 of 624) were outside the customary 70 to 120% (13 were below 70% and 13 above 120%).
When the average method spike recovery for each analytical set is examined (average of iprodione, isomer, and metabolite) the range was 61 to 119% with only 3 average recoveries below 70%; rice straw at 12 months (61%), garlic at 9 months (64%), and rice grain at 12 months (68%).
The method spike recovery data averaged across substrates ranged from 68 to 112%. When averaged compounds the mean recovery was 90% for iprodione, 89% for the isomer and 101% for the metabolite.
These data demonstrate that the results obtained were within acceptable limits. The analyses conducted for this study continued for more than 18 months. The method spike data observed were what would be expected for such an extended period of time.

II. RESULTS AND DISCUSSION

The stability results are expressed as average percentage of the nominal fortification and are not corrected for the procedural recoveries. In order to account for possible variations over the time investigated, the mean procedural recovery results are given in addition.
The mean recoveries were the actual percent of the residues recovered in the stored samples based on the amount of iprodione, the isomer and the metabolite added to control samples. These recoveries give insight into the validity of the uncorrected residues reported in RAC and processing studies when stored under similar conditions. Corrected recoveries in stored samples equate to actual loss of residues during storage. Corrections were made for the experimental variability observed in the method spikes that were analyzed with the stored samples. Corrected recovery values above 70% were considered to indicate that the residues were stable.
Both the mean recovery and corrected recovery in the stored samples for most substrates were above 70% for all three compounds when stored under frozen conditions for 12 months or more. Some of the substrates had mean recoveries below 70%, each of which is discussed below.
Oilseed rape seed: The mean recovery of isomer RP-30228 at 12 months averaged at 55%. After correction for the procedural recovery, the average recovery was 68%. The other compounds were stable for the entire 12 months.
Oilseed rape crude oil: The mean recoveries that were below 70% were for the 3-month iprodione and the 3-, 6- and 9-month RP-30228 samples. After correcting for the respective procedural recovery, 81%, 83% and 82% result for RP-30228, respectively. Only 69% result for iprodione; however, the results for the later sampling dates were above 70% which suggests that the recoveries below 70% were due to analytical variation and that the residues are stable for 12 months in oilseed rape crude oil.
Carrot: The 28-month mean recovery was 66% for RP-30228. Correction for the procedural recovery resulted in an average of 86%.
Garlic: Several of the mean recoveries were below 70%. However, these lower values are directly related to the method spike recovery values. Analysis for iprodione and RP-30228 in garlic was very difficult as indicated by the lower method spike recovery values. Comparison of the apparent recovery results for each compound show that the actual values were somewhat low; the residues remained constant during the 12-month storage time which indicates that the residues are stable for at least 12 months. The corrected recoveries were all above the acceptable 70% for all sampling intervals.
Ginseng: The 12-month mean recovery was 66% for metabolite RP-32490. Correction for the procedural recovery resulted in an average of 100% for the corrected recovery. Thus, the low RP-32490 apparent recoveries were due to analytical variation and not loss during storage.
Raisins: The mean recoveries were below 70% for the 6-month iprodione and the 12-month RP-30228 samples. After correcting for the respective procedural recovery, 72% and 86% result, respectively.
Raisin waste: Mean recoveries of iprodione were below 70% after three months. However, after the initial decline to about 67% at three months, the recoveries remained constant. The average corrected recoveries iprodione were between 69% and 77% for the 3- to 9-month samples. The same trend was observed for the 3- and 6-month RP-30228 samples. The data for the RP-32490 samples were all above 70%.

Peanut vines: The mean recoveries were below 70% for the 3-month iprodione and the 6-month RP-30228 samples. After correcting for the respective procedural recovery, 76% and 80% result, respectively. Mean recoveries after longer storage intervals show that the residues of both compounds are stable for at least 12 months.
Peanut meal: Mean recoveries were above 70% except for the 3-month iprodione with 61%. Correction for the procedural recovery resulted in 74%.
Peanut crude oil: Mean recoveries were above 70% except for the 6-month RP-30228 samples with 68%. The average recovery corrected for the procedural recovery was 88%.
Peanut soapstock: Analysis of iprodione, RP-30228 and RP-32490 in peanut soapstock was not possible. The high pH of the soapstock resulted in rapid degradation of these compounds. Consistent measurable residues were not possible. It is unlikely that any residues resulting from commercial practices would result in measurable residues in peanut soapstock.
Potato granules: Mean recoveries were above 70% except for the 6-month RP-30228 samples with 69%. Correction for the procedural recovery resulted in 76%.
Rice grain: Most of the mean recoveries were below 70%. However, these lower values are directly related to the method spike recovery values. Comparison of the apparent recovery results for each compound show that the actual values were somewhat low, the residues remained constant during the 12-month storage time which indicates that the residues are stable for at least 12 months. The corrected recoveries were all above the acceptable 70% for all sampling intervals.
Rice straw: The mean recovery of the 6-month RP-30228 samples was 69% (75% when corrected for the procedural recovery). The results for the 12-month samples for all compounds are questionable. No additional contingency sample sets were available to reanalyze for the 12-storage interval. However, the remaining data indicate that all three compounds are stable in rice straw under frozen conditions for at least nine months.

These results indicate that iprodione, its isomer and its metabolite are stable frozen in most plant matrices for at least 12 months. The results from the extended storage stability study lengthen that storage stability period, because the data obtained clearly indicate that iprodione, its isomer and its metabolite are stable for 24 to 34 months when stored under frozen conditions. Any variations of results fell within normal expectations for analyses of residue substrates.

The tables below present a summary of the recoveries from the stored fortified samples:

Table 6.1-1 Storage stability of iprodione (BAS 610 F) in plant matrices

Mean Recovery (%)										
	A: in stored samples, % of nominal				B: procedural, in freshly spiked sample					
Months	**A**	B	**A**	B	**A**	B	**A**	B	**A**	B
	Almond hulls		**Almond nutmeat**		**Apple**		**Blueberry**		**Broccoli**	
0	**95**	94	**92**	94	**92**	119	**84**	85	**91**	93
3	**83**	95	**82**	91	**83**	88	**87**	97	**95**	94
6	**82**	98	**78**	91	**74**	79	**75**	83	**85**	91
9	**82**	91	**76**	92	**77**	86	**77**	85	**104**	109
12	**84**	85	**81**	89	**85**	90	**79**	95	**91**	92
30/29	**91**	84	**82**	93	-	-	-	-	-	-
	OSR presscake		**OSR seed**		**OSR crude oil**		**OSR refined oil**		**Carrot**	
0	**91**	92	**125** (107)	116	**83**	93	**109**	99	**86**	87
3	**87**	88	**92**	101	**67** (69)	98	**88**	96	**86**	86
6	**111**	102	**124** (139)	89	**81**	101	**88**	87	**87**	90
9	**102**	97	**87**	91	**74**	87	**94**	100	**88**	96
12	**93**	94	**77**	72	**85**	104	**99**	95	**90**	95
28	-	-	-	-	-	-	-	-	**95**	102
	Maize grain		**Cotton seed**		**Cucumber**		**Garlic**		**Ginseng root**	
0	**92**	93	**106**	105	**102**	101	**79**	74	**78**	95
3	**90**	95	**99**	90	**88**	98	**67** (93)	76	**89**	91
6	**88**	94	**94**	89	**n.r.**	n.r.	**80**	73	**91**	92
9	**81**	91	**96**	88	**88**	94	**77**	61	**92**	91
12	**86**	95	**94**	94	**87**	92	**67** (96)	70	**77**	80
21	-	-	-	-	**91**	88	-	-	-	-
33/30	**87**	90	**89**	80	-	-	-	-	-	-
	Grapes		**Dry grape pomace**		**Wet grape pomace**		**Raisins**		**Raisin waste**	
0	**85**	88	**105**	103	**94**	92	**96**	95	**87**	96
3	**92**	93	**80**	94	**92**	98	**84**	80	**67** (77)	86
6	**90**	89	**86**	92	**89**	77	**67** (72)	93	**66** (69)	95
9	**90**	85	**88**	100	**84**	95	**73**	81	**61** (69)	88
12	**86**	88	**84**	82	**95**	101	**80**	84	**71**	94
15	**92**	93	-	-	-	-	-	-	-	-
	Grape juice		**Lettuce**		**Onion dry bulb**		**Peaches**		**Peanut hay**	
0	**89**	88	**98**	90	**106**	107	**95**	96	**92**	104
3	**88**	92	**86**	84	**101**	99	**111**	90	**98**	105
6	**86**	91	**89**	89	**87**	89	**87**	93	**101**	107
9	**84**	84	**92**	96	**88**	92	**92**	86	**88**	79
12	**82**	86	**93**	91	**77**	80	**86**	92	**81**	80
34	-	-	**92**	91	-	-	-	-	-	-

Table 6.1-1 Storage stability of iprodione (BAS 610 F) in plant matrices

Mean Recovery (%)										
	A: in stored samples, % of nominal				B: procedural, in freshly spiked sample					
Months	**A**	B	**A**	B	**A**	B	**A**	B	**A**	B
	Peanut hulls		**Peanut nutmeat**		**Peanut vines**		**Peanut meal**		**Peanut crude oil**	
0	**70**	85	**89**	87	**75**	80	**88**	89	**91**	87
3	**83**	94	**90**	93	**53** (76)	70	**61 (74)**	83	**86**	86
6	**70**	89	**97**	94	**81**	66	**88**	93	**83**	90
9	**84**	86	**93**	97	**87**	90	**88**	93	**86**	88
12	**81**	81	**89**	86	**85**	90	**93**	93	**91**	93
	Peanut refined oil		**Peanut soapstock**		**Peppers**		**Potato chips**		**Potato granules**	
0	**87**	81	**n.r.**	n.r.	**92**	85	**96**	99	**88**	85
3	**91**	91	**n.r.**	n.r.	**87**	95	**85**	80	**77**	84
6	**88**	89	**n.r.**	n.r.	**92**	95	**90**	96	**73**	94
9	**95**	96	**n.r.**	n.r.	**89**	91	**87**	95	**82**	92
12	**88**	88	**n.r.**	n.r.	**93**	93	**92**	91	**77**	90
	Rice bran		**Rice grain**		**Rice hulls**		**Polished rice**		**Rice straw**	
0	**91**	85	**61** (90)	67	**86**	88	**86**	98	**97**	94
3	**90**	96	**66** (90)	72	**129** (158)	82	**89**	92	**76**	89
6	**92**	96	**66** (73)	90	**96**	101	**87**	92	**80**	105
9	**87**	88	**65** (74)	89	**85**	92	**91**	92	**81**	78
12	**98**	103	**67** (97)	69	**95**	93	**92**	95	**57** (134)	43
	Strawberry		**Tobacco**		**Tomato**					
0	**92**	95	**73**	72	**97**	106				
3*	**94**	95	**75**	79	**84**	80				
6	**92**	98	**n.r.**	n.r.	**90**	67				
9	**90**	92	**n.r.**	n.r.	**91**	97				
12	**92**	92	**n.r.**	n.r.	**91**	95				
24	**-**	-	**-**	-	**92**	84				

() Values in parenthesis were corrected for procedural recovery
OSR Oilseed rape
n.r. Not reported
* 10 weeks in case of tobacco

Table 6.1-2 Storage stability of isomer RP-30228 in plant matrices

Mean Recovery (%)										
	A: in stored samples, % of nominal				B: procedural, in freshly spiked sample					
Months	**A**	B	**A**	B	**A**	B	**A**	B	**A**	B
	Almond hulls		**Almond nutmeat**		**Apple**		**Blueberry**		**Broccoli**	
0	**80**	82	**90**	94	**112**	115	**76**	77	**96**	85
3	**94**	117	**82**	88	**73**	90	**75**	95	**93**	88
6	**75**	99	**80**	91	**77**	71	**74**	82	**77**	84
9	**88**	90	**70**	90	**91**	98	**70**	87	**81**	94
12	**107**	85	**77**	93	**78**	89	**81**	100	**81**	89
30/29	**86**	82	**73**	90	-	-	-	-	-	-
	OSR presscake		**OSR seed**		**OSR crude oil**		**OSR refined oil**		**Carrot**	
0	**89**	88	**89**	89	**71**	84	**89**	88	**84**	81
3	**84**	88	**97**	126	**68** (81)	84	**84**	93	**92**	86
6	**86**	112	**77**	83	**69** (83)	83	**75**	82	**79**	82
9	**84**	89	**71**	87	**62** (82)	76	**92**	99	**79**	83
12	**86**	89	**55** (68)	81	**86**	82	**87**	85	**112**	121
28	-	-	-	-	-	-	-	-	**66** (86)	77
	Maize grain		**Cotton seed**		**Cucumber**		**Garlic**		**Ginseng root**	
0	**90**	90	**87**	86	**84**	87	**66** (97)	67	**82**	77
3	**86**	90	**91**	90	**100**	106	**60** (85)	71	**81**	91
6	**85**	93	**97**	95	**n.r.**	n.r.	**93**	84	**118**	118
9	**83**	92	**83**	73	**88**	97	**75**	46	**74**	77
12	**82**	96	**106**	95	**82**	93	**75**	71	**84**	79
21	-	-	-	-	**86**	90	-	-	-	-
33/30	**89**	90	**84**	87	-	-	-	-	-	-
	Grapes		**Dry grape pomace**		**Wet grape pomace**		**Raisins**		**Raisin waste**	
0	**75**	78	**105**	102	**93**	95	**96**	91	**88**	94
3	**85**	93	**74**	81	**85**	97	**86**	82	**59** (68)	87
6	**83**	86	**70**	92	**88**	78	**82**	92	**63** (70)	89
9	**76**	71	**76**	89	**82**	99	**72**	85	**70**	88
12	**88**	92	**99**	93	**76**	94	**66** (86)	77	**71**	94
15	**86**	87	-	-	-	-	-	-	-	-
	Grape juice		**Lettuce**		**Onion dry bulb**		**Peaches**		**Peanut hay**	
0	**72**	74	**94**	87	**84**	111	**94**	95	**110**	115
3	**77**	85	**84**	86	**88**	97	**84**	92	**89**	106
6	**79**	88	**84**	91	**87**	93	**98**	90	**82**	99
9	**77**	81	**86**	94	**95**	95	**92**	92	**89**	93
12	**72**	70	**86**	92	**92**	100	**87**	93	**72**	76
34	-	-	**91**	93	-	-	-	-	-	-
	Peanut hulls		**Peanut nutmeat**		**Peanut vines**		**Peanut meal**		**Peanut crude oil**	
0	**85**	77	**94**	88	**78**	81	**85**	92	**91**	88
3	**76**	86	**76**	91	**76**	91	**73**	93	**77**	84
6	**92**	102	**82**	91	**67** (80)	83	**78**	94	**68** (88)	77
9	**69** (91)	75	**85**	90	**80**	85	**80**	91	**78**	73
12	**82**	89	**80**	82	**79**	94	**83**	91	**77**	85

Table 6.1-2 Storage stability of isomer RP-30228 in plant matrices

Mean Recovery (%)										
	A: in stored samples, % of nominal				B: procedural, in freshly spiked sample					
Months	**A**	B	**A**	B	**A**	B	**A**	B	**A**	B
	Peanut refined oil		**Peanut soapstock**		**Peppers**		**Potato chips**		**Potato granules**	
0	**84**	79	**n.r.**	n.r.	**88**	84	**96**	97	**90**	84
3	**88**	93	**n.r.**	n.r.	**85**	90	**84**	77	**77**	83
6	**87**	91	**n.r.**	n.r.	**91**	94	**89**	93	**69** (76)	91
9	**95**	87	**n.r.**	n.r.	**85**	90	**84**	92	**82**	94
12	**87**	91	**n.r.**	n.r.	**90**	93	**89**	86	**82**	89
	Rice bran		**Rice grain**		**Rice hulls**		**Polished rice**		**Rice straw**	
0	**89**	85	**62** (79)	78	**79**	83	**93**	91	**81**	74
3	**94**	92	**68** (94)	73	**83**	105	**86**	91	**71**	64
6	**100**	101	**65** (77)	84	**82**	102	**86**	95	**69** (75)	93
9	**117**	97	**76**	93	**87**	97	**87**	89	**94**	104
12	**100**	116	**60** (97)	62	**91**	104	**93**	95	**92**	41
	Strawberry		**Tobacco**		**Tomato**					
0	**87**	93	**81**	82	**91**	101				
3*	**89**	89	**80**	82	**76**	87				
6	**89**	93	**n.r.**	n.r.	**75**	83				
9	**78**	94	**n.r.**	n.r.	**89**	108				
12	**86**	95	**n.r.**	n.r.	**87**	94				
24	**-**	-	**-**	-	**87**	83				

() Values in parenthesis were corrected for procedural recovery
OSR Oilseed rape
n.r. Not reported
* 10 weeks in case of tobacco

Table 6.1-3 Storage stability of metabolite RP-32490 in plant matrices

Mean Recovery (%)										
	A: in stored samples, % of nominal				B: procedural, in freshly spiked sample					
Months	**A**	B	**A**	B	**A**	B	**A**	B	**A**	B
	Almond hulls		**Almond nutmeat**		**Apple**		**Blueberry**		**Broccoli**	
0	**132** (100)	131	**111**[1]	114	**88**	113	**95**	95	**96**	97
3	**94**	99	**100**	104	**94**	94	**99**	113	**98**	104
6	**94**	99	**94**	100	**112**	112	**108**	119	**87**	92
9	**90**	91	**89**	96	**102**	114	**86**	87	**108**	115
12	**103**	110	**92**	96	**93**	95	**82**	94	**111**	113
30/29	**92**	92	**97**	103	-	-	-	-	-	-
	OSR presscake		**OSR seed**		**OSR crude oil**		**OSR refined oil**		**Carrot**	
0	**103**	111	**118**	125	**103**	117	**104**	107	**99**	105
3	**84**	92	**111**	129	**88**	106	**93**	95	**95**	85
6	**93**	104	**100**	97	**97**	99	**102**	106	**89**	87
9	**97**	92	**90**	91	**80**	92	**99**	101	**111**	115
12	**111**	104	**94**	100	**120**	126	**88**	90	**116**	125
28	-	-	-	-	-	-	-	-	**98**	102
	Maize grain		**Cotton seed**		**Cucumber**		**Garlic**		**Ginseng root**	
0	**80**	109	**119**	116	**90**	97	**93**	85	**100**	88
3	**110**	113	**99**	101	**96**	102	**104**	104	**106**	102
6	**107**	111	**101**	100	**n.r.**	n.r.	**100**	94	**109**	114
9	**96**	99	**101**	100	**91**	103	**79**	86	**92**	83
12	**82**	91	**103**	109	**93**	98	**88**	82	**66** (100)	66
21	-	-	-	-	**88**	92	-	-	-	-
33/30	**93**	95	**89**	93	-	-	-	-	-	-
	Grapes		**Dry grape pomace**		**Wet grape pomace**		**Raisins**		**Raisin waste**	
0	**94**	97	**116**	117	**99**	99	**104**	101	**89**	102
3	**100**	105	**103**	119	**121** (93)	129	**115**	102	**101**	106
6	**95**	96	**93**	89	**96**	86	**94**	92	**81**	93
9	**107**	110	**101**	104	**89**	101	**89**	94	**88**	102
12	**102**	104	**98**	90	**112**	112	**88**	88	**90**	96
15	**90**	95	-	-	-	-	-	-	-	-
	Grape juice		**Lettuce**		**Onion dry bulb**		**Peaches**		**Peanut hay**	
0	**93**	93	**90**	83	**95**	102	**91**	92	**99**	113
3	**99**	104	**110**	112	**107**	93	**85**	88	**97**	100
6	**92**	96	**109**	118	**113**	114	**113**	118	**87**	83
9	**100**	100	**85**	108	**93**	107	**95**	94	**86**	79
12	**108**	109	**95**	99	**91**	99	**92**	101	**78**	76
34	-	-	**93**	100	-	-	-	-	-	-
	Peanut hulls		**Peanut nutmeat**		**Peanut vines**		**Peanut meal**		**Peanut crude oil**	
0	**84**	84	**93**	97	**83**	84	**111**	115	**104**	104
3	**114**	119	**91**	99	**72**	97	**78**	82	**95**	100
6	**96**	79	**86**	94	**74**	68	**90**	96	**93**	96
9	**84**	82	**103**	108	**130** (94)	138	**102**	106	**99**	106
12	**71**	71	**98**	99	**88**	98	**96**	99	**97**	104

Table 6.1-3 Storage stability of metabolite RP-32490 in plant matrices

Mean Recovery (%)										
	A: in stored samples, % of nominal				B: procedural, in freshly spiked sample					
Months	**A**	B	**A**	B	**A**	B	**A**	B	**A**	B
	Peanut refined oil		**Peanut soapstock**		**Peppers**		**Potato chips**		**Potato granules**	
0	**97**	91	**n.r.**	n.r.	**108**	102	**117**	115	**101**	99
3	**97**	103	**n.r.**	n.r.	**102**	102	**116**	104	**92**	93
6	**92**	93	**n.r.**	n.r.	**103**	108	**97**	104	**92**	100
9	**109**	113	**n.r.**	n.r.	**96**	96	**94**	105	**97**	105
12	**99**	103	**n.r.**	n.r.	**109**	110	**100**	101	**101**	110
	Rice bran		**Rice grain**		**Rice hulls**		**Polished rice**		**Rice straw**	
0	**102**	100	**64 (78)**	82	**138** (100)	139	**95**	99	**105**	100
3	**117**	124	**74**	77	**95**	104	**96**	100	**119**	109
6	**110**	115	**74**	88	**90**	102	**89**	92	**99**	108
9	**81**	99	**81**	90	**104**	102	**110**	108	**82**	91
12	**97**	102	**69** (95)	73	**125**	115	**123** (102)	121	**116**	98
	Strawberry		**Tobacco**		**Tomato**					
0	**94**	89	**107**	101	**92**	101				
3*	**82**	93	**77**	76	**83**	86				
6	**113**	122	**n.r.**	n.r.	**116**	104				
9	**104**	107	**n.r.**	n.r.	**88**	96				
12	**88**	96	**n.r.**	n.r.	**96**	104				
24	**-**	-	**-**	-	**88**	88				

() Values in parenthesis were corrected for procedural recovery
OSR Oilseed rape
n.r. Not reported
* 10 weeks in case of tobacco
1 Value of 36% where portion of sample was lost during workup was not considered

Standard solutions of the test and external standard compounds have been shown to be stable for periods in excess of six months when stored below -10°C. Standard solution stability was demonstrated for this study by weighing out new aliquots of iprodione, its isomer and its metabolite, preparing new standard dilutions and comparing to the previously weighed standard solutions. This was done approximately every six months of the total study duration. All standard comparisons showed variations of less than 10% which is the limit normally accepted for high pressure liquid chromatography.

III. CONCLUSION

Overall, residues of iprodione, its isomer and its metabolite are stable when stored at -10°C for up to 12 months. Residues of all three compounds were not stable in peanut soapstock because of the high pH of this substrate. There were no general trends indicating that any of the residues were declining with time. The results varied within what would be expected to be normal analytical variability with residue analyses over several months. The results from the extended storage stability study lengthen that storage stability period, because the data obtained clearly indicate that iprodione, its isomer and its metabolite are stable for 24 to 34 months when stored under frozen conditions. Any variations of results fell within normal expectations for analyses of residue substrates.

The following study also containing the active ingredient Vinclozolin was added to support the storage stability of the common metabolite with Iprodione, 3,5-dichloroaniline as discussed. The data for soil is added for completeness reasons only.

Report:	CA 6.1/3 Portnoy C.E.,Horton W.E., 1980a Freezer storage stability of BAS 352 F and three of its 3,5-dichloroaniline-containing metabolites in strawberries and soil 1980/10133
Guidelines:	none
GLP:	no

Executive Summary

A freezer storage stability study was conducted for vinclozolin (BAS 352 F) and three of its 3,5-dichloroaniline-containing metabolites in strawberries and soil. Samples were fortified with the compounds at 0.2 and 5.0 mg/kg; soil samples were fortified with vinclozolin and two of its metabolites only. All samples were stored frozen at -15°C and analyzed at various intervals. As only metabolite 3,5-dichloroaniline is relevant for iprodione, the other compounds are not reported any further.
Residues of 3,5-dichloroaniline in strawberries and soil remained essentially unchanged after freezer storage of 29 and 27 months, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Vinclozolin (BAS 352 F), BF 352-31 (Metabolite D; 3,5-dichloroaniline),
BF 352-23 (Metabolite E), BF 352-22 (Metabolite B)

Description:

Lot/Batch #: Not relevant

Purity: Not reported

CAS#: 9002-04-4 (vinclozolin)

Spiking levels: 0.2 mg/kg, 5.0 mg/kg

2. Test Commodity:

Crop: Strawberry, soil

Sample size: 40-50 g (fortified samples)

B. STUDY DESIGN AND METHODS

1. Test procedure

A freezer storage stability study was conducted for 3,5-dichloroaniline in strawberries and soil. Samples were fortified at 0.2 and 5.0 mg/kg. All samples were stored frozen at -15°C. Strawberry samples were analyzed after 3, 6, 9, 13, 19 and 29 months; soil samples were analyzed after 3, 6, 9, 12, 16 and 27 months.

2. Description of analytical procedures

The fortified strawberry and soil samples were analyzed at each interval according to BWC Agricultural Chemicals method No 25. The fortified plant matrices were hydrolyzed directly and the dichloroaniline was extracted from the aqueous solution and then derivatized with chloroacetylchloride. The derivative was pure enough for quantitation by GC.

II. RESULTS AND DISCUSSION

Residues of 3,5-dichloroaniline in strawberries and soil remained essentially unchanged after freezer storage of 29 and 27 months, respectively. Recoveries were between 70 and 120% except in soil after three months.

Table 6.1-4 Storage stability of 3,5-dichloroaniline in strawberries and soil

Mean Recovery (%)				
Months	0.2 mg/kg	5.0 mg/kg	0.2 mg/kg	5.0 mg/kg
	Strawberry		**Soil**	
Metabolite D (3,5-dichloroaniline)				
3	119	72	65	n.r.
6	112	75	81	n.r.
9	95	97	70	n.r.
12	-	-	98	n.r.
13	n.r.	71	-	-
16	-	-	109	70
19	105	n.r.	-	-
27	-	-	n.r.	80
29	124	78	-	-

n.r. Not reported

III. CONCLUSION

No significant losses of 3,5-dichloroaniline occurred in fortified strawberry or soil samples after storage in excess of 24 months.

Report:	CA 6.1/4 Enders C., 2013a Interim report: Investigation of storage stability of Reg. No. 85831 and Reg. No. 5079612 in plant matrices 2013/1311892
Guidelines:	EEC 7032/VI/95 rev. 5, EEC 1607/VI/97 rev. 2 10.06.1999, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EPA 860.1380,OECD 506
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

A freezer storage stability study was performed investigating several plant samples spiked with iprodione metabolites RP 32596 and RP 37176. The study is planned for a period of 730 days. This interim report gives the results from 0 to 545 days.
The samples were separately spiked with the test items at concentration levels of 0.2 mg/kg. The spiked samples were stored under the usual storage conditions for field samples (about -18°C in the dark) and analyzed after different intervals.
Samples were analyzed with BASF method No L0180/01, which allows the quantitation of residues of BAS 610 F and its metabolites RP 32490, RP 30228, RP 32596, RP 37176 and M610F007 to a limit of 0.01 mg/kg in plant matrices. The samples were analyzed for metabolites with LC-MS/MS. Procedural recoveries averaged at 102% (RP 32596) and 104% (RP 37176), respectively.
The data indicate that metabolite RP 37176 is stable when stored at -18°C for up to 545 days. The mean recovery is above 70%. Metabolite RP 32596 was stable in dried bean seed. In lettuce leaves the mean recovery after this storage time (545 days) was 21.7%, for carrots roots 16.5% and for strawberry fruits 56.5%. Rapeseed stated very good recoveries (91.7%) up to 342 days storage time. At the latest time point measured (542 days) recovery decreased to 67%. A further assessment needs to be made when the final completion of the interim report is available.

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	Iprodione metabolites RP 32596 and RP 37176 (Reg.No.5079612)
	Description:	
	Lot/Batch #:	L33-79, FRH1727
	Purity:	99.8% (RP 32596), 97.2% (RP 37176)
	CAS#:	626-43-7 (RP032596)
	Spiking levels:	0.2 mg/kg
2.	**Test Commodity:**	
	Crop:	Lettuce (leaves), carrots (roots), strawberry (fruits), dried bean (seed), rapeseed (seed)
	Sample size:	Not specified

B. STUDY DESIGN AND METHODS

1. Test procedure

The stability of two metabolites of iprodione, RP 32596 and RP 37176 was investigated under deep frozen conditions (-18°C) over a time period of 539 to 542 days in lettuce leaves, carrot roots, strawberry fruits, dried bean seed and rapeseed seed.
The samples were spiked with the test items at a concentration level of 0.2 mg/kg. The spiked samples were stored frozen and analyzed after different intervals (0, 30, 90, 180, 345, 545 and 730 days.). This summary gives the interim results after about 545 days. Results after about 730 days will be given in the final report.

2. Description of analytical procedures

The method used for the analysis of all substrates was BASF method No L0180/01 by means of LC-MS/MS. The limit of quantitation (LOQ) of the method was 0.01 mg/kg.
The metabolites were extracted with acetonitrile and water using a Polytron by approximately 8000 rpm. A portion of the extract is centrifuged and an aliquot of the supernatant is diluted for determination by LC-MS/MS.
Method recoveries were analyzed with each set of stored samples.
The overall range of individual recovery values for the metabolite RP 32596 was 91 to 118% with a mean of 102 ± 5.3%. For RP 37176 overall range of individual recovery values was 91 to 113%. Thus, values were within 70 to 120%.
Data demonstrate that the results obtained were within acceptable limits. The analyses conducted for this study continued for about 545 days.

II. RESULTS AND DISCUSSION

The stability results are expressed as average percentage of the nominal fortification. Mean recoveries were the actual percent of the residues recovered in the stored samples based on the amount of the metabolites added to control samples.
For RP 37176, the mean recovery of the nominal fortification in all stored samples were above 70% when stored under frozen conditions for about 540 days and therefore show stability in all matrices.
Concerning metabolite RP 32596, the stability was demonstrated for dried bean seed for about 540 days and for rapeseed up to 342 days storage time. For lettuce leaves the mean recovery after this storage time was 21.7%, for carrots roots 16.5%, for strawberry fruits 56.5%. The mean recovery of rapeseed was 91.7% up to 342 days storage time. The latest time point (542 days) was at 67% recovery. Further time points will show if it is an exception or a starting instability.

The tables below present a summary of the recoveries from the stored fortified samples:

Table 6.1-5 Storage stability of RP 32596 in plant matrices

Mean Recovery (%)										
	A: in stored samples, % of nominal B: procedural, in freshly spiked sample									
Days	**A**	B	**A**	B	**A**	B	**A**	B	**A**	B
	Lettuce Leaves		**Carrots Roots**		**Strawberry Fruits**		**Dried Bean Seed**		**Rapeseed Seed**	
0	**104**	98.8	**101**	99.1	**106**	104	**123**	116	**109**	107
28	**70.3**	106	**59.6**	109	**91.5**	105	**110**	101	**103**	99.7
55-59	**53.3**	101	**42.5**	119	**83.1**	99.5				
85-87	**57.1**	102	**45.2**	99.9	**83.8**	101	**109**	105	**105**	106
175-182	**37.6**	104	**21.9**	99.0	**70.9**	99.6	**103**	102	**95.8**	101
339-342	**24.8**	98.0	**17.5**	99.4	**69.0**	97.3	**103**	101	**91.7**	97.9
539-542	**21.7**	98.8	**16.5**	97.4	**56.5**	96.9	**96.2**	103	**67.0**	95.1

Table 6.1-6 Storage stability of RP 37176 in plant matrices

Mean Recovery (%)										
	A: in stored samples, % of nominal B: procedural, in freshly spiked sample									
Days	**A**	B	**A**	B	**A**	B	**A**	B	**A**	B
	Lettuce Leaves		**Carrots Roots**		**Strawberry Fruits**		**Dried Bean Seed**		**Rapeseed Seed**	
0	**107**	116	**110**	113	**108**	105	**107**	102	**101**	102
28	**107**	111	**108**	101	**110**	103	**95.0**	88.1	**99.2**	98.4
85-87	**107**	109	**107**	109	**111**	109	**122**	112	**115**	110
175	**108**	111	**111**	107	**109**	104	**95.9**	91.7	**106**	100
339-342	**92.5**	95.0	**101**	103	**99.6**	104	**96.3**	106	**96.2**	105
539-542	**102**	108	**107**	110	**99.4**	102	**106**	101	**102**	98.9

III. CONCLUSION

Overall, residues of RP 37176 are stable in all plant matrices when stored at -18°C for up to 542 days. Residues of RP 32596 are stable in dried bean seed, but not in lettuce leaves, carrots roots and strawberry fruits after this storage time. In rapeseed very good recoveries were found up to 342 days storage time. At the latest time point (542 days) the stability decreased to 67%.
For lettuce and strawberry the storage stability was demonstrated for 28 days and for at least 182 days, respectively.

Discussion and overall conclusion

In addition to the active substance Iprodione the stability of its isomer (RP30228) and its metabolite (RP32490) in various commodities and processing fractions with high water, high acid, high oil, high starch content as well as dry matrices was investigated (CA 6.1/1-2). Since no trials on commodities with high protein content are submitted, the submission of a storage stability study on these matrices was not considered necessary. The results are summarized in table 7.1-7.

Table 6.1-7: Summary table storage stability data for Iprodione and its metabolites RP 30228 and RP 32490 in plant matrices based on CA 6.1/1-2

Commodity	Timeframe covered	Category
Almond Hulls	30 months	Dry commodity
Almond Nutmeat	29 months	High oil
Apple	12 months	High water
Blueberry	12 months	High acid
Broccoli	12 months	High water
OSR presscake	12 months	Dry commodity
OSR seed	12 months	High oil
OSR crude oil	12 months	High oil
OSR refined oil	12 months	High oil
Carrot	28 months	High starch
Maize grain	33 months	High starch
Cotton seed	30 months	High oil
Cucumber	21 months	High water
Garlic	12 months	High water
Ginseng root	12 months	Difficult to analyse
Grapes	15 months	High acid
Dry grape pomace	12 months	Dry commodity
Wet grape pomace	12 months	Difficult to analyse
Raisins	12 months	High acid
Raisin waste	12 months	Difficult to analyse
Grape juice	12 months	High acid
Lettuce	34 months	High water
Onion dry bulb	12 months	High water
Peaches	12 months	High water
Peanut hay	12 months	Dry commodity
Peanut hull	12 months	Dry commodity
Peanut nutmeat	12 months	High oil
Peanut vines	12 months	High water
Peanut meal	12 months	Dry commodity
Peanut crude oil	12 months	High oil
Peanut refined oil	12 months	High oil
Peanut soapstock	12 months	Difficult to analyse
Peppers	12 months	High water
Potato chips	12 months	High starch, high oil
Potato granules	12 months	High starch
Rice bran	12 months	Dry commodity
Rice grain	12 months	High starch
Rice hulls	12 months	Dry commodity
Polished rice	12 months	High starch
Rice straw	12 months	Dry commodity
Strawberry	12 months	High acid
Tobacco	12 months	Difficult to analyse
Tomato	24 months	High water

For RP32596 (3,5-dichloroaniline) storage stability data in strawberries specifically (as a common metabolite with Vinclozolin) is presented in CA 6.1/3.

A new freezer storage stability study (CA 6.1/4) was started for RP37176 and RP32596 (3,5-dichloroaniline) in commodities with high water (salad), high acid (strawberry), high oil (rape seed), high protein (dried beans) and high starch (carrot roots) content planned for up to 24 month. So far stability in all categories was demonstrated for RP37176 for a period of up to 545 days at -20 °C.

Within CA 6.1/4 stability for RP32596 (3,5-dichloroaniline) at -20 °C could be shown for up to 342 and 545 days only in dried beans and rape seed, respectively. Low recoveries were obtained for all matrices containing significant amount of water such as salad, strawberry and carrot roots.

It is assumed that the instability seen in this study is not representative for incurred residues but a result of an interaction of the test substance dried onto the homogenized sample material and the re-solubilisation during sample extraction. This assumption could be confirmed during non GLP experiments. Additionally, a high rate of conjugation for 3,5-dichloroaniline was also observed in the plant metabolism studies (CA 6.2) confirming the high reactivity of this molecule.
This assumption is also supported by a separate storage stability study for RP32596 (3,5-dichloroaniline) in strawberries (CA 6.1/3) stored at -15°C that showed good stability for up 29 months.
Within a recent residue study (CA 6.3.1/6, 2014/1049029) lettuce samples were reanalysed for RP32596 after a significant additional storage time of up to 1596 days. In the few cases that 3,5 dichloroaniline was detected the results from both analysis compared very well not indicting an instability under frozen conditions.
It is therefore concluded that the set-up of the recent storage stability study (CA 6.1/4) for RP32596 (3,5-dichloroaniline) in matrices with significant water content is impacting the recovery observed and not relevant for incurred residues. Additionally, a degradation of the 3,5-dichloroaniline moiety is chemically also not to be expected.

Based on the finding of various conjugates from 3,5-dichloroaniline in the plant metabolism study as described in CA 6.2 as well as results from residue trials (CA 6.3) it is unlikely that a storage stability study on incurred residues is feasible due to the low residues present.

For M610F007 (Reg. No 5916256) a new freezer storage stability study in plant matrices is planned but was only recently started (assigned BASF Doc ID: 2013/1311887).

The provided data is also designed to also cover the data requested by EFSA in 2013 (Reasoned opinion, 2013;11(10):3438).

Since no additional animal feeding studies are submitted, the submission of a stability study of residues in products of animal origin is not considered necessary.

In previously submitted feeding studies no information on the storage stability of iprodione or its hydroxylated and non-hydroxylated metabolites in milk, muscle, fat, liver and kidney, eggs was reported. However, a storage stability study is not required as the residue definition includes all metabolites containing the 3,5-dichloroaniline and 3,-5-dichloro-4-hydroxy moieties and degradation of these moieties is not expected (EFSA Reasoned opinion, 2013;11(10):3438).

CA 6.2 Metabolism, distribution and expression of residues

CA 6.2.1 Metabolism, distribution and expression of residues in plants

In the framework of the original inclusion into Annex I according to Directive 91/414/EEC as well as during the re-evaluation according to Article 12 of Regulation (EC) No 396/2005 the following studies investigating of the metabolism of the active substance iprodione in plant were peer-reviewed: on fruits and fruiting vegetables (peach and strawberry), leafy vegetables (lettuce), pulses and oilseeds (peanuts) and cereals (rice and wheat). Studies were done using ^{14}C-phenyl-labelled iprodione and before the GLP requirement became applicable. The characteristics of these studies are summarized in Table 6.2.1-1.

Table 6.2.1-1: Summary of metabolism studies in plants previously available

Group	Crop	Method, F or G (a)	Rate (kg a.s./ha)	No	Sampling (DAT)	Year	DocID
Fruits and fruiting vegetable	Peach	Foliar, F	1.1	3	pre-2nd appl., pre-3rd appl., 8 DALA	1981	C022388
	Strawberry	Foliar, F	1	1	7, 14, 32	1977	C022424
			2	2	7, 14, 21, 28		
		Soil spray (before sowing), G	4	1	35, 61, 93, 125		
			10		36		
Leafy vegetables	Lettuce	Foliar and soil spray to run off, G	0.75	1	0, 16, 25, 38	1981	C022495
Pulses and oilseeds	Peanut	Foliar, F	1.1	3	3h-post 1st appl., pre/post 2nd appl., pre/post 3rd appl., 10 DALA	1983	C023023
Cereals	Rice	Foliar, F	1.1	2	9 x up to harvest	1983	R003014
	Wheat	Foliar, G	1	1	0, 7, 15, 33, 70, 96	1977	C022424
		Soil spray (before sowing), G	1	1	30, 59, 77		
			10	1	16, 44, 89		

(a): Outdoor/field application (F) or glasshouse/protected/indoor application (G)

After foliar application on **peach**, the parent compound represented almost all the residues recovered in the fruits (94 % of the TRR) at harvest. The other identified compound, RP30228, accounted for 0.8 % of the TRR, whereas the metabolite RP32490 was only estimated at 0.5 % (due to poor resolution).

In **strawberry** the major amount of the applied radioactivity (97%) was recovered in leaves and stem while only 0.5% was recovered in fruits. At the higher application rate, parent iprodione was reported to be the main component of the TRR in leaves (78 % TRR) and fruits (56 % TRR) at harvest. In considerably lower levels metabolites RP30228 (leaves 5% TRR, fruit 3% TRR) and RP32490 (leaves 3% TRR, fruits 1.6% TRR) were found at harvest. The remainder of the residues in fruit was classed as non-identified products in the organic phase (34 % TRR) and bound products (4.1% TRR).

In **lettuce,** 38 days after treatment, parent iprodione was found at 81% of the TRR, metabolite RP30228 accounted for 9.5 % of the TRR respectively. The non-identified and bound parts of TRR amounted to 4.5% each.

In **peanuts**, the most significant residue was unchanged iprodione, accounting for about 43% and 54% and of the TRR in hulls and hay, respectively. In hulls, no individual metabolite was present at a level greater than 6 % of TRR. In hay, RP30228 and RP32490 accounted for up to 15% and 9% of the TRR, respectively. In addition, metabolites RP36112 and RP25040 could be identified in this study occurring up to 7% TRR (one exception: immature plant, day 31, RP36112 27% total residue). A large portion of the residues remained unidentified or could not be extracted (about 14% of the TRR in hay and 45% of the TRR in hulls). In hulls, the radioactivity was too low for further characterisation (<0.05 mg/kg).

Also in **rice**, parent was found in high and RP30228 in low amounts (86% / 6% of TRR) at day 0. In the course of the study RP30228 increased compared to parent. Metabolites RP25040 was also present in amounts up to 9% of TRR. RP32490 and RP36112 could not be separated. Like in other studies the amount of bound residues was increasing to up to 26% of TRR in polished rice at harvest.

In **wheat** at day 0, the major part of the residue in leaf and stems was formed by the parent compound (94 % of TRR) followed by lower amounts of the metabolites RP30228 and RP32490 (1.2 / 1.5 % of TRR). During the study a shift of the ratio parent / RP30228 towards the metabolite was observed (day 96: parent 25%, RP30228 33 %, RP32490 1.2 % of TRR).
A large portion of the residues remained unextractable (up to 28 % of the TRR in leaves and stems). In wheat heads, recovered radioactivity was very low (0.16 %), therefore, extracted residues remained unidentified or bound.

After **soil treatment** with iprodione, the uptake by plants was reported to be low (1-5 %) and the compound appeared to be extensively degraded to form RP30228. Other metabolites such as RP32490 and more polar unidentified products were found in small amounts. A relatively high portion of the residues (up to 38-55 % on strawberries and 50 % on wheat) remained not extracted.

The metabolism studies performed between 1977 and 1983 show essential similarities: In all cases unchanged parent iprodione was reported to be the major compound, followed by its rearranged isomer RP30228. The ratio of these two components was shifted towards the metabolite during the studies in all cases. Metabolite RP32490 was found in all studies, however in varying amounts up to 9% of TRR (one exception 14%). In the oldest studies no further metabolites could be assigned, in the later ones metabolites RP36112 and RP25040 were found, mostly below 6% of TRR.

All metabolites which were identified in the reported plant metabolism studies are shown in Figure 6.2.1-3. Although not uniform throughout the studies, the findings do not suggest major differences regarding metabolic pathways in these various crops belonging to different categories.
The main degradation reactions are

- cleavage of the dioxoimidazolidine ring followed by isomerization to form RP30228
- desalkylation under formation of RP32490
- further stepwise degradation of both possible heterocyclic rings / the biuret unit

All studies have in common that considerable amounts of the total radioactive residue could not be identified or remained unextracted. The reason for incomplete identification is certainly the relatively low quality of analytical equipment of the 1970s to mid 1980s. The thin layer chromatography used in this time was not potent and sensitive enough to detect minor components in very low absolute residue amounts.

The tendency of iprodione to form unextractable bound residues is a common finding independent of the plant species investigated. Attempts to further solubilize the bound residues have not been done in the past.

Against this background it was intended to perform one more metabolism study in order to confirm the results as shown above and agreed upon so far and to further clarify the remaining unidentified amounts with state-of-the-art analytic technology as well as with more drastic solubilization reactions in order to investigate the bound portions.

Considering the crop groups covered by metabolism studies as well as the intended uses it was decided to conduct a new metabolism study in carrot as representative of the root and tuber vegetables.

Report:	CA 6.2.1/1 Sowka N. et al., 2014a Metabolism of ^{14}C-Iprodione in carrots 2012/1220412
Guidelines:	OECD 501 - Metabolism in crops (adopted January 8 2007), EPA 860.1000: 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), JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The objective of the study in carrots was to investigate the amount of iprodione (BAS 610 F) residues and the nature of iprodione degradation products after foliar application. The carrots were treated four times with a mixture of ^{14}C-labeled and unlabeled iprodione at a nominal rate of 750 g a.s./ha. The first application was carried out 58 days before harvest and the last application 28 days before harvest.

For both carrot root and leaf, soluble radioactive residues were predominantly extracted with methanol (root: 62.6% TRR and leaf: 96.8% TRR) and only minor amounts were subsequently released with water (≤1.7% TRR). Solubilization of the residues after solvent extraction with ammonia and enzymes released additional 3.9% (root) and 1.0% (leaf) of the TRR.

High amounts of iprodione parent was detected in carrot leaf (48.458 mg/kg, 80.6% TRR), whereas parent residues in carrot root were significantly lower (0.037 mg/kg, 7.6% TRR). Hence, iprodione was extensively metabolized in carrot root.

In carrot root, the glutamic acid conjugate M610F007 was the most abundant component (16.4% TRR). Metabolite M610F001 is an isomer of the parent compound, which might partially be a work-up artefact. In carrot root, the sum of iprodione and M610F001 was 21.6% of the TRR. The remaining identified components (carbohydrates, M610F009 / others, M610F006, M610F005, M610F011, M610F003 and M610F002 ranged from 1.2% to 6.1% of the TRR. In carrot leaf, the sum of iprodione and M610F001 was 95.0% of the TRR and only small portions of carbohydrates, M610F006, M610F011, M610F003 and M610F002 were identified (≤1.1% TRR).

The metabolism of iprodione includes, cleavage of the acetate moiety of the imidazolinedione ring, cleavage of the isopropyl group (dealkylation), successive degradation of the biuret side chain (hydrolysis of the amine group, decarboxylation) and conjugation of metabolites with glutamic acid, serine, glycine, malonic acid and glucose. The degradation products of iprodione are eventually incorporated into carbohydrates.
Carrot root and leaf were also extracted with methanol only (residue method L0180/01), with acetone/water/2 M HCl (70/25/5, residue method 543/0) and acetonitrile (QuEChERS method). The extractabilities with methanol only and acetone/water/2 M HCl were similar to those obtained by the methanol and water extractions of the metabolism investigations. The metabolite patterns and amount of metabolites were also similar to the results of the solvent extracts of the metabolism investigations. Although the extractability with acetonitrile (QuEChERS method) was somewhat lower, the metabolite pattern and the amount of metabolites were generally comparable to the results of the metabolism investigations. Particularly the sum of the parent compound and its isomer M610F001 was virtually identical for all extraction methods.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: Phenyl-U-^{14}C-iprodione; unlabeled iprodione (BAS 610 F)
Lot/Batch #: 967-1001 (^{14}C); MD2085
Purity: Radiochemical purity: 95.3%
Chemical purity: 94.9% (^{14}C); 99.8%
Specific activity: 6.69 MBq/mg
CAS#: 36734-19-7

Stability of test compound: The test item was stable over the test period.

2. Test Commodity:

Crop: Carrot
Type: Root and tuber vegetables
Variety: Nantaise 2, Topfix JW
Botanical name: *Daucus carota*
Crop parts(s) or processed commodity: Roots, leaves
Sample size: Not relevant

3. Soil: A sandy loam soil was used. The soil physicochemical properties are described below (see Table 6.2.1-2).

Table 6.2.1-2: Soil physicochemical properties

Soil Series	Soil Type	pH	TOC %	Sand %	Silt %	Clay %	Maximal water holding capacity	CEC[1] cmol/kg
Bruch West*	Sandy loam**	7.2**	1.85***	69.1*	21.6*	9.2*	33.4	12.3

* USDA scheme ** ($CaCl_2$) *** Total organic carbon 1 Cation exchange capacity

B. STUDY DESIGN AND METHODS

The study was carried out at the Agricultural Center of BASF SE in Limburgerhof, Germany. The objective was to investigate the amount of iprodione (BAS 610 F) residues and the nature of iprodione degradation products after foliar application to carrots. The cultivation of the carrots took place under natural climatic conditions without the influence of rain in vegetation hall.

1. Test procedure

Carrot plants were treated four times with a spray application of radiolabeled iprodione (BAS 610 F) at a nominal application rate of 750 g a.s./ha (approximately 0.67 Ib/A). The first application was carried out at a PHI of 58 days, the second application was performed at a PHI of 48 days, the third at 38 days and the last application was carried out 28 days before harvest.
Carrot leaves and roots were stored deep frozen at or below -18°C until analysis; extracts were stored refrigerated or frozen, depending on the storage period.

2. Description of analytical procedures

TRR combusted: For the determination of the TRR combusted, homogenized plant material was weighed and combusted by means of a sample oxidizer.

Extraction: Aliquots of homogenized plant material were extracted three times with methanol. The methanol extracts of the three steps were combined and measured by LSC. The residue was further extracted with water (twice). The water extracts were also combined and radio-assayed. The combined results of methanol extractions and water extractions are referred to as extractable radioactive residues (ERR).

The residue after solvent extraction of each sample was dried and homogenized. Aliquots were combusted for the determination of the residual radioactive residue (RRR).

RRR after solvent extraction: The residue after solvent extraction of carrot root and leaf was solubilized twice in 1% ammonia. Thereafter, the residue was dried, homogenized and re-suspended in acetate buffer. The residue after macerozyme / cellulase solubilization was dried, re-suspended in acetate buffer and incubated in the presence of hesperidinase and glucosidase. Thereafter, the residue was re-suspended in phosphate buffer and α-amylase, β-amylase and amyloglucosidase were added. The residue after amylase solubilization of carrot root was re-suspended in artificial gastric juice. Thereafter, the residue was re-suspended in artificial intestinal fluid.

HPLC analyses: The **root** methanol extract was concentrated and analyzed with HPLC. The concentrated methanol extract was also used for co-chromatography experiments with the reference item iprodione. A similar concentrated methanol extract was used for co-chromatography with the reference items iprodione and M610F001 [RP30228]. The root water extract was concentrated, filtrated and analyzed with HPLC. A concentrated root methanol extract was also analyzed with HPLC for storage stability purposes.

The **leaf** methanol extract was concentrated and analyzed with HPLC. The concentrated extract was centrifuged and the supernatant was analyzed with HPLC. The concentrated methanol extract was also used for co-chromatography experiments with the reference items iprodione and M610F001 [RP30228]. The leaf water extract was concentrated and analyzed with HPLC. The same water extract was also used for co-chromatography experiments with the reference items iprodione and M610F001 [RP30228]. The concentrated ammonia solubilizate of leaf and the concentrated macerozyme solubilizate of leaf were analyzed with HPLC.

Isomerization: The degree of isomerization of the parent compound iprodione to M610F001 [RP30228] was investigated after different storage intervals in methanol, acetonitrile, acetonitrile/water (80/20), acetone and acetone/water/2 M HCl (70/25/5). For each test, the reference item iprodione was evaporated to dryness and re-dissolved in one of the solvents listed above. The solutions were analyzed using HPLC immediately after solvent addition, after several hours and after storage of the samples for up to 1-4 days at room temperature.

Work-up of alternate solvent extractions: In addition to the solvent extractions with methanol and water, carrot root and leaf samples were also extracted with the following alternative extraction protocols:

Residue method L0180/01: The samples were mixed with methanol, extracted with a homogenizer and centrifuged. The extracts were concentrated and analyzed using HPLC.

Residue method 543/0: The samples were mixed with a mixture of acetone/water/2 M HCl (70/25/5), extracted with a homogenizer and centrifuged. The extracts were concentrated and analyzed using HPLC.

QuEChERS method: The samples were mixed with acetonitrile, extracted with a homogenizer and centrifuged. The supernatant was decanted and adjusted to a volume of 50 mL (in case of leaf) with a mixture of acetonitrile/water (75/25). The extracts were concentrated and analyzed using HPLC.

3. Identification of metabolites

Isolation of metabolites from carrot roots: The root methanol extract was concentrated to the water phase and spiked with methanol and acetonitrile. After solvent partitioning, the ethyl acetate phases were dried over sodium sulfate. The combined ethyl acetate was concentrated and subsequently small amounts of methanol, acetonitrile, water and detergent were added. The concentrated sample was fractionated and subsequently purified with HPLC. The purified fractions were subjected to mass spectrometric analysis.
The water phase after ethyl acetate partition was concentrated and fractionated using HPLC, whereby two peaks were cut. The polar fraction was concentrated and used for co-chromatography experiments with the reference item ^{14}C-sucrose using HPLC.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

The total radioactive residue (TRR) was calculated by summarizing the extractable radioactive residues (ERR) and the residual radioactive residue (RRR) after solvent extraction. The calculated TRR of carrot root was 0.482 mg/kg and the calculated TRR of carrot leaf was 60.132 mg/kg. For both matrices the calculated TRR was set to 100% TRR.

Table 6.2.1-3: Total radioactive residues (TRRs) in carrot samples following foliar application of ^{14}C-iprodione

TRRs in treated carrot root and leaf			
Matrix	**DALA**	**TRR determined by direct combustion [mg/kg]**	**TRR calculated [mg/kg][1]**
Root	28	0.467	0.482
Leaf	28	63.667	60.132

DALA = days after last application
[1] Sum of ERR (extraction with methanol and water) and RRR

B. EXTRACTION, CHARACTERIZATION AND IDENTIFICATION OF RESIDUES

1. Extraction of residues

The extractability was 64.4% TRR for carrot root and 98.0% TRR for carrot leaf. For both matrices the major part of the radioactivity was extracted with methanol (root: 62.6% TRR and leaf: 96.8% TRR) and only minor amounts were subsequently released with water (root: 1.7% TRR and leaf: 1.1% TRR).
The residual radioactive residue of carrot root (0.172 mg/kg or 35.6% TRR) and carrot leaf (1.228 mg/kg or 2.0% TRR) was solubilized with ammonia, macerocyme, hesperidinase, amylase, pepsin (only root) and pancreatin (only root). Thereby, 3.9% TRR of carrot root and 1.0% TRR of carrot leaf were additionally released.

The extractabilities of radioactive residues from carrot roots and leaves are summarized in Table 6.2.1-4.

Table 6.2.1-4: Extraction efficiency of residues of ^{14}C-iprodione in carrot samples

Matrix	TRR calc.*	Distribution of radioactive residues				ERR[1]		RRR[2]	
		Methanol extract		Aqueous extract					
	[mg/kg]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Root	0.482	0.302	62.6	0.008	1.7	0.310	64.4	0.172	35.6
Leaf	60.132	58.228	96.8	0.676	1.1	58.904	98.0	1.228	2.0

* TRR was calculated as the sum of ERR + RRR
1 ERR = extractable radioactive residue
2 RRR = residual radioactive residue (after solvent extraction)

2. Identification, characterization and quantitation of extractable residues

Structure elucidation of metabolites was based on mass spectrometric analysis of isolated fractions of carrot root. HPLC-MS analysis of the fractions led to the identification of the parent compound iprodione and its metabolites M610F001 [RP30228], M610F002 [RP36221], M610F005 [RP36115], M610F006 [RP44247], M610F007, M610F003 [RP36112], M610F004 [RP25040], M610F008, M610F009, M610F011 and M610F010. Metabolite M610F001 [RP30228] is an isomer of the parent compound, of which the imidazolinedione ring is rearranged. Stability experiments in different solvents indicated that M610F001 [RP30228] might partially be a work-up artefact.
In carrot **root**, metabolites M610F007 and M610F001 [RP30228] were the most abundant components and accounted for 0.079 mg/kg or 16.4% TRR and 0.067 mg/kg or 14.0% TRR, respectively. Carbohydrates, M610F006 [RP44247], M610F005 [RP36115], M610F011 and the unchanged parent compound iprodione were present from 0.016 mg/kg or 3.2% TRR to 0.037 mg/kg or 7.6% TRR. The remaining identified components (M610F009 / others, M610F003 [RP36112] and M610F002 [RP36221]) were below or equal to 0.006 mg/kg or 1.3% TRR. In the ERR of carrot root 0.293 mg/kg or 60.7% TRR were identified and additional 0.007 mg/kg or 1.4% TRR were characterized by HPLC. The RRR of carrot root was characterized by solubilization.

In carrot **leaf**, the most abundant component was the parent compound iprodione, which accounted for 48.458 mg/kg or 80.6% TRR. Metabolite M610F001 [RP30228] was also present at significant amounts (8.673 mg/kg or 14.4% TRR). Moreover, small amounts of carbohydrates, M610F006, M610F011 and M610F002 [RP36221] were detected (up to 1.1% TRR). In carrot leaf 58.245 mg/kg or 96.9% TRR were identified and additional 1.663 mg/kg or 2.8% TRR were characterized.

Table 6.2.1-5: Metabolites detected in carrot matrices following foliar applications of ^{14}C-iprodione

Components	Root		Leaf	
	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Carbohydrates	0.016	3.2	0.137	0.2
M610F007	0.079	16.4	-	-
M610F009 / others	0.006	1.3	-	-
M610F006 [RP44247]	0.029	6.0	0.053	0.1
M610F005 [RP36115]	0.029	6.1	-	-
M610F011	0.017	3.6	0.238	0.4
M610F003 [RP36112]	0.006	1.3	0.012	<0.1
Iprodione	0.037	7.6	48.458	80.6
M610F002 [RP36221]	0.006	1.2	0.675	1.1
M610F001 [RP30228]	0.067	14.0	8.673	14.4
Total identified	**0.293**	**60.7**	**58.245**	**96.9**
Total characterised	0.007	1.4	1.663	2.8
Total identified and/or characterized	**0.299**	**62.1**	**59.908**	**99.6**
Final residue	0.172	35.6	0.357	0.6
Grand total	**0.471**	**97.7**	**60.265**	**100.2**

3. Proposed metabolic pathway

The proposed metabolic pathway of iprodione in carrot is shown in Figure 6.2.1-1. Iprodione (BAS 610 F) is extensively metabolized and degradation products are eventually incorporated into carbohydrates. The metabolism of iprodione includes the following reactions: (a) cleavage of the acetate moiety of the imidazolinedione ring, (b) cleavage of the isopropyl group (dealkylation), (c) successive degradation of the biuret side chain (hydrolysis of the amine group, decarboxylation) and (d) conjugation of metabolites with glutamic acid, serine, glycine, malonic acid and glucose.

4. Extractability of residues according to residue analytical methods

The extractabilities with methanol only (root: 57.5% TRR and leaf: 91.4% TRR) and acetone/water/2 M HCl (root: 59.7% TRR and leaf: 95.4% TRR) were similar to those obtained by the methanol and water extractions of the metabolism investigations. The metabolite patterns and amount of metabolites were also similar to the results of the solvent extracts of the metabolism investigations. Although the extractability with acetonitrile (QuEChERS method) was somewhat lower (root: 40.2% TRR and leaf: 77.2% TRR), the metabolite pattern and the amount of metabolites were generally comparable to the results of the metabolism investigations. Particularly the sum of the parent compound and its isomer M610F001 was virtually identical for all extraction methods.

Table 6.2.1-6: Extraction efficiency of residues of ^{14}C-iprodione in carrot samples with alternate extraction solvents

Matrix	Methanol (residue method L180/01)		Acetone/water/2 M HCl (residue method L543/0)		Acetonitrile (QuEChERS method)	
	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Root	0.286	57.5	0.301	59.7	0.193	40.2
Leaf	61.403	91.4	63.309	95.4	48.875	77.2

5. Storage stability

The solvent extracts (methanol and water) of carrot leaf were extracted and HPLC analyzed within a maximum of 162 days. The solvent extractions for quantitation of carrot root residues were carried out later, for which reason the sampling to analysis intervals were up to 292 days. However, the metabolite pattern of a concentrated methanol extract of an early extraction (28 days after sampling), which was obtained 6 days after extraction with HPLC, was virtually identical to the metabolite pattern of the quantitative analysis. Hence, the corresponding metabolites were stable throughout the period of investigation and no additional storage stability experiments were necessary.

III. CONCLUSION

After foliar application of iprodione (BAS 610 F) on carrots (approximately 750 g a.s./ha) for four times, high amounts of total radioactive residues were detected in carrot leaf (60.132 mg/kg), whereas TRRs in carrot root were significantly lower (0.482 mg/kg).

For both matrices, soluble radioactive residues were predominantly extracted with methanol (root: 62.6% TRR and leaf: 96.8% TRR) and only minor amounts were subsequently released with water (≤1.7% TRR). Solubilization of the residues after solvent extraction with ammonia and enzymes released additional 3.9% (root) and 1.0% (leaf) of the TRR.

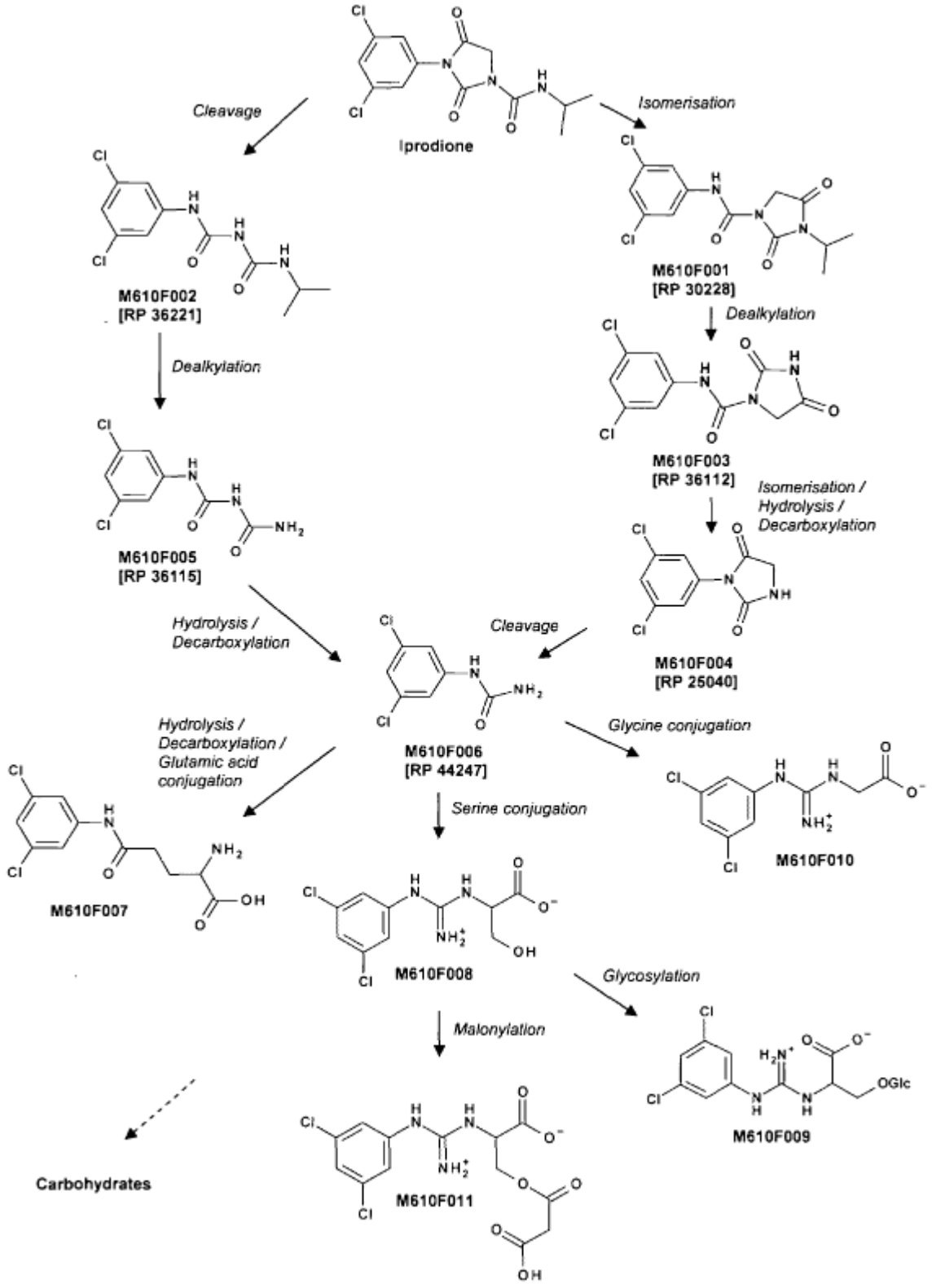
In carrot root, the glutamic acid conjugate M610F007 was the most abundant component (16.4% TRR). Metabolite M610F001 [RP30228] is an isomer of the parent compound (imidazolinedione rearranged), which might partially be a work-up artefact. In carrot root, the sum of iprodione and M610F001 [RP30228] was 21.6% of the TRR. The remaining identified components (carbohydrates, M610F009 / others, M610F006 [RP44247], M610F005 [RP36115], M610F011, M610F003 [RP36112] and M610F002 [RP36221] ranged from 1.2% to 6.1% of the TRR.

In carrot leaf, the sum of iprodione and M610F001 [RP30228] was 95.0% of the TRR and only small portions of carbohydrates, M610F006 [RP44247], M610F011, M610F003 [RP36112] and M610F002 [RP36221] were identified (≤1.1% TRR).

The metabolism of iprodione includes the following reactions: (a) cleavage of the acetate moiety of the imidazolinedione ring, (b) cleavage of the isopropyl group (dealkylation), (c) successive degradation of the biuret side chain (hydrolysis of the amine group, decarboxylation) and (d) conjugation of metabolites with glutamic acid, serine, glycine, malonic acid and glucose. The degradation products of iprodione are eventually incorporated into carbohydrates.

Carrot root and leaf were also extracted with methanol only (residue method L0180/01), with acetone/water/2 M HCl (70/25/5, residue method 543/0) and acetonitrile (QuEChERS method). The extractabilities with methanol only and acetone/water/2 M HCl were similar to those obtained by the methanol and water extractions of the metabolism investigations. The metabolite patterns and amount of metabolites were also similar to the results of the solvent extracts of the metabolism investigations. Although the extractability with acetonitrile (QuEChERS method) was somewhat lower, the metabolite pattern and the amount of metabolites were generally comparable to the results of the metabolism investigations. Particularly the sum of the parent compound and its isomer M610F001 was virtually identical for all extraction methods.

Figure 6.2.1-1: Proposed metabolic pathway of iprodione in carrot



Report: CA 6.2.1/2
Fujisawa T. et al., 2006a
Publication: Uptake and transformation of pesticide metabolites by duckweed (Lemna gibba)
2006/1050853
Guidelines: none
GLP: no

This entry was taken from public literature und not included in the application.

Executive Summary

Uptake and transformation of ^{14}C-labeled 3,5-dichloroaniline (DCA) were examined by using duckweed (Lemna gibba). It was observed that DCA underwent phase II conjugation with glucose and glutamic acid. The identities of the conjugates were confirmed by various spectrometric analyses and/or HPLC co-chromatography with reference synthetic standards. The tests were performed in comparison with metabolites of other pesticides which are not included in this summary.
The results confirm the formation of iprodione metabolite M610F007 (in the carrot study CA 6.2.1) the identity of which was proved by spectroscopic data in this article. The findings contribute to the understanding of possible reactions of DCA in plants.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:
   Description: [phenyl-^{14}C] 3,5-dichloroaniline
   Purity: Radiochemical purity: >97%
   Specific activity: 5 GBq/mmol
   CAS#: 626-43-7

2. Test Commodity:
   Plant: Duckweed
   Botanical name: *Lemna gibba*

B. STUDY DESIGN AND METHODS

The work was carried out at Environmental Health Science Laboratory, Sumitomo Chemical Company, Japan. The objective was to investigate uptake and transformation of ^{14}C-labeled 3,5-dichloroaniline after application to duckweed. The duckweed plants were maintained in pots filled with a water/sediment system collected from the paddy field. The plants were grown in a greenhouse equipped with a quartz glass ceiling, and the temperature was kept at 25 °C.

1. Test procedure

0.1 g/mL aqueous solutions (100 mL) of [^{14}C]-DCA was prepared and diluted with corresponding non-radiolabeled reference standard to give a total radioactivity of ca. 83 kBq (5 000 000 dpm) in exposure water. A 3 g sample of duckweed was exposed to the chemical, and sampling of plants and exposure water was conducted on days 1, 2, and 4. During the incubation, the mass of duckweed increased finally to 4.3-5.5 g. Sampling was conducted during the fourth day of exposure. Samples were divided into duckweed and exposure water and stored in a freezer until analysis.

2. Description of analytical procedures

Duckweed samples were cut and extracted with an acetone/water mixture (4:1, v/v). After work-up plant extract and exposure water were radio-assayed by LSC and analyzed by HPLC.

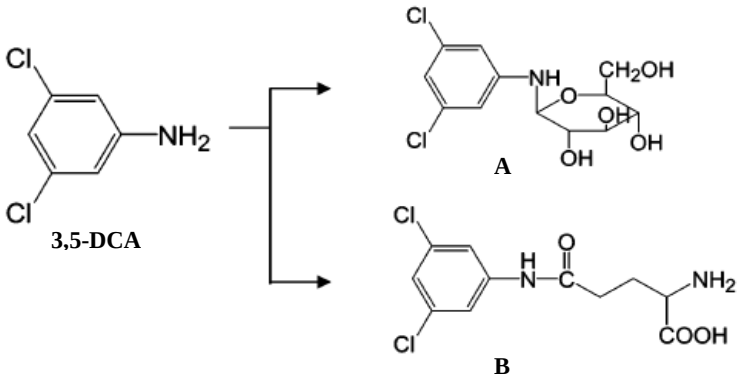
3. Results and Discussion

Recovery of Radioactivity: From the results of uptake experiments, a good recovery of 119.8% of [^{14}C]-DCA from the test system was observed during the incubation.

Uptake: The radioactivity taken up by duckweed was estimated by combining extractable and unextractable ^{14}C (<1% of TRR). An uptake of 70.1% of ^{14}C was observed reaching a plateau after 4 days.

Identification of Metabolites: 3,5-Dichloroaniline was metabolized to the N-glucoside A (51% TRR) and the glutamate conjugate **B** (24% TRR) which is identical with M610F007. 25% of TRR remained unchanged as DCA. The metabolite structures were confirmed by HPLC co-chromatography with the synthetic as well as by LC-APCI-MS and LC-ESI-MS analyses in positive and negative ion modes.

Figure: Transformations of 3,5-Dichloroaniline in Duckweed



4. Conclusion

The investigation of the metabolism of 3,5-dichloroaniline in Duckweed *Lemna gibba* showed that phase I metabolism such as oxidation and reduction was not detected. Instead, phase II conjugations were observed with 3,5-DCA clearly preferring glucose and glutamic acid as reaction partner. The other test substances not included here also reacted with glucose but also with different small organic acid derivatives.

Report: CA 6.2.1/3
Fujisawa T. et al., 2009a
Publication: Application of separated leaf cell suspension to xenobiotic metabolism in plant
2009/1130762
Guidelines: none
GLP: no

This entry was taken from public literature und not included in the application.

Executive Summary

Primarily, this publication describes a separated leaf cell suspension system which was found suitable to reproduce the in vivo plant metabolism. Though the method as such is not relevant for this dossier, it uses 3,5-dichloroaniline as example and therefore contributes to the understanding of 3,5-dichloroaniline metabolism in plants.

The metabolic profiles of ^{14}C-labeled 3,5-dichloroaniline was examined by using enzymatically separated leaf cell suspension from seedlings of cabbage (Brassica oleracea) and tomato (Lycopersicon esculentum). After 1 day of incubation, the metabolite was extensively transformed in cabbage, whereas it was scarcely metabolized in tomato. The major metabolic pathways were the phase II reactions leading to conjugates such as glucoside and glutamate. The chemical identities of the secondary metabolites were determined by spectrometric analyses and/or HPLC co-chromatography with the synthetic reference standards.
The results show that metabolite M610F007 which was in the iprodione metabolism study in carrot (CA 6.2.1) only detected in root can also be formed in other crops and in other plant parts.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:
   Description: [phenyl-^{14}C] 3,5-dichloroaniline
   Purity: Radiochemical purity: >97%
   Specific activity: 2.22 GBq/mmol
   CAS#: 626-43-7

2. Test Commodity:
   Plant: 1) cabbage 2) tomato
   Botanical name: 1) *Brassica oleracea* 2) *Lycopersicon esculentum*

B. STUDY DESIGN AND METHODS

The work was carried out at Environmental Health Science Laboratory, Sumitomo Chemical Company, Japan. The main objective was to test a system of separated leaf cell suspension for in-vitro investigation of plant metabolism. The test system itself, its advantages in comparison to other techniques and the other examples used are not of interest in this dossier and therefore not included in this summary.

In order to prove the usefulness of this method ^{14}C-labeled 3,5-dichloroaniline was applied to separated leaf cell suspensions of cabbage and tomato and the resulting metabolic profile was investigated.

1. Test procedure

Seeds of cabbage and tomato were sown and grown under controlled conditions. Samples were taken at the forth leaf stage. From these leaves separated leaf cell suspensions were prepared which were treated with a solution of the test compound [^{14}C]-DCA. The system was kept at 25°C in dark. Samples were taken after 1, 2 and 4 days of exposure.

2. Description of analytical procedures

The incubated samples were first divided into leaf cells and culture medium by filtration followed by multi-step work-up and purification. Finally, analyses were done by LSC and HPLC.

3. Results and Discussion

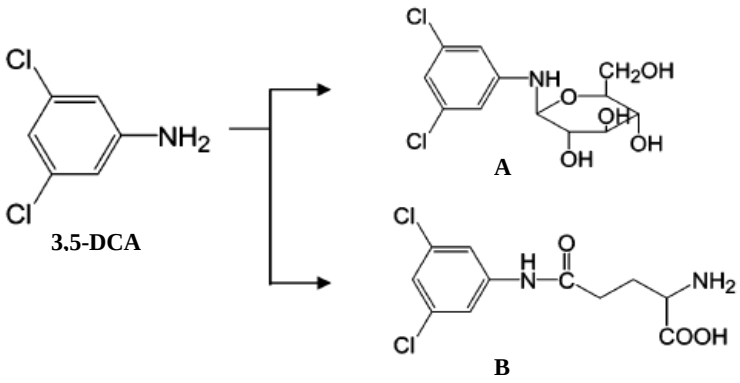
Recovery of Radioactivity: From the results of uptake experiments, a good recovery of 80.6% of [^{14}C]-DCA from the test system was observed after 1 days of incubation.

Identification of Metabolites: In cabbage, 3,5-Dichloroaniline was completely metabolized forming the N-glucoside A (24% TRR) and the glutamate conjugate **B** (16% TRR). In tomato, only the glucoside A (15% TRR) was found and 70% of TRR remained unchanged as DCA. The metabolite structures were confirmed by HPLC co-chromatography with the synthetic as well as by LC-APCI-MS and LC-ESI-MS analyses in positive and negative ion modes.

Table 6.2.1-7: ^{14}C Distribution Cell Suspension System

Portion		Metabolite	% TRR	
			cabbage	tomato
leaf cell ^{14}C	extractable		47.8	79.0
		3,5-DCA	nd	63.9
		A	23.7	15.1
		B	16.3	nd
	unextractable		3.0	5.3
medium ^{14}C			49.2	15.7
		3,5-DCA	49.2	15.7

Figure 6.2.1-2: Transformation of 3,5-Dichloroaniline in Cabbage



4. Conclusion

As main aim of this work it could be shown that the conjugation reactions observed in vivo were also found in the separated leaf cell suspension. Also a species difference known from metabolism studies in vivo was considered to be reflected in this test system.
With special regard to 3,5-dichloroaniline it was demonstrated that in cabbage leaf cell suspension a quantitative transformation took place within a day.

Overall Summary Plant Metabolism

The present Annex I renewal dossier provides a new metabolism study in carrot as representative for root and tuber vegetables. The new study mainly confirms the findings and degradation reactions known so far and complements additional details due to more sensitive technology and further treatment of the bound residues.
Combining the findings of the older metabolism studies and the new one, a complex system of equilibriums and degradation reactions results which can not only explain most of the facts gathered so far but also lead to a more complete picture (see also Figure 6.2.1-3):

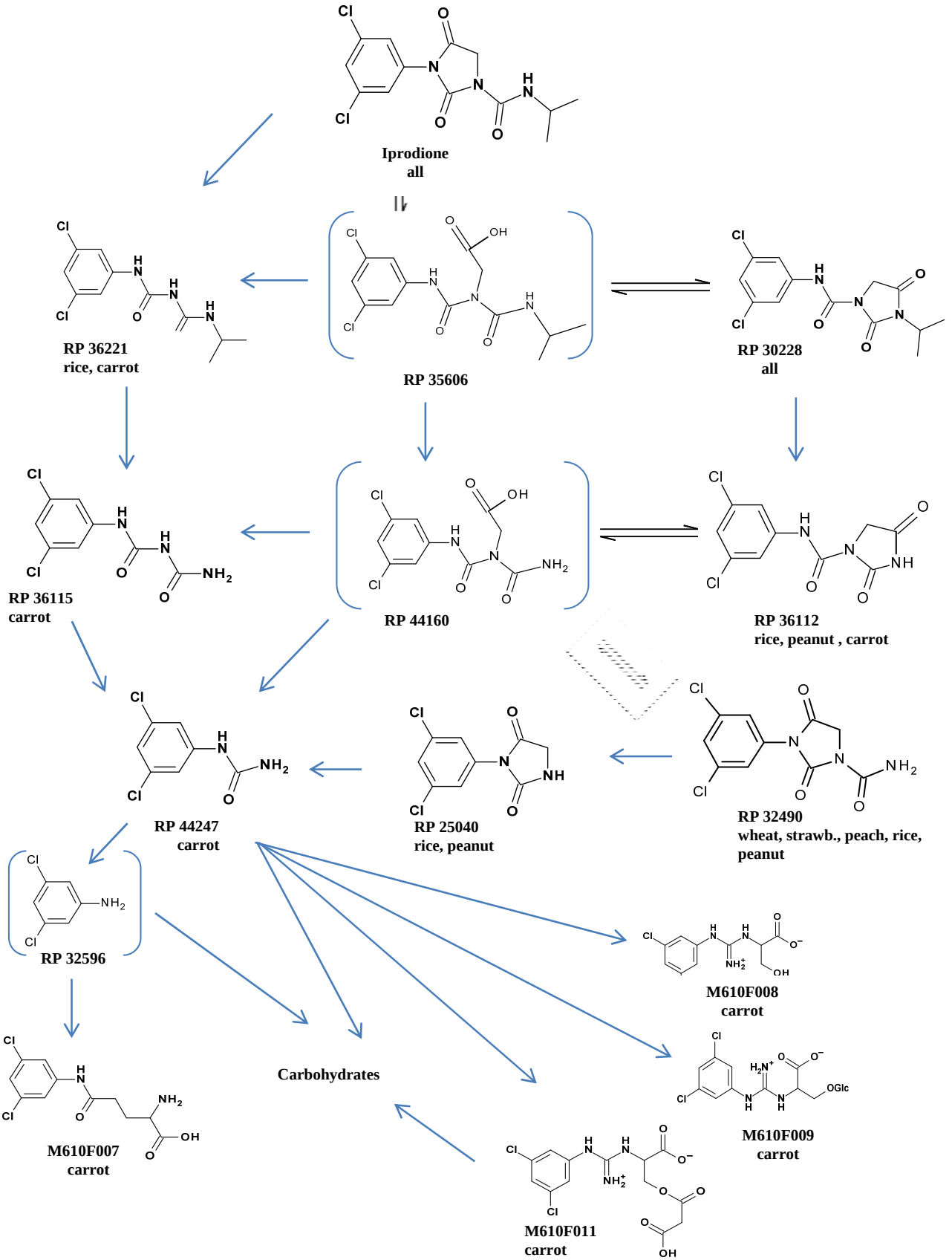
- Common in all metabolism studies is the unique transformation of parent iprodione by ring-opening of the heterocyclic ring and rearrangement to form its constitutional isomer RP30228.
- Already in the summary of the old studies it was stated that the metabolism in the different crops showed qualitative similarities but quantitative differences. This is confirmed further: like the pH-depending equilibrium of parent / RP35606 / RP30228 (see CA 7.2.1.1/1) a comparable relation exists after removal of the isopropyl group between RP32490 / RP44160 / RP36112 which can be understood as explanation for the varying amounts of RP 32490 and RP 36112 in the different crops.
- It can be assumed that the degradation route via the open chain intermediates RP36221 and RP36115 now found in carrot does principally also occur in other crops but was not detected in the old studies due to very small amounts.
- The finding of conjugates between 3,5-dichloroaniline or urea-derivative RP44247 and naturally occurring amino acids (M610F007 to M610F011) can be understood as examples for the amounts of extracted but unidentified compounds in the old studies. In addition to these metabolites now described a wide variety of combinations between iprodione derived urea units and amino acids is possible.
- The new metabolism study in carrot shows that the degradation products of iprodione are finally incorporated into carbohydrates which in case of high molecular structures explains the high amount of bound residues which were found in all crops investigated.

In addition to the facts which are in accordance with the old studies, a new outcome of the metabolism study in carrot is the finding of the conjugate between 3,5-dichloroaniline (DCA) and glutamic acid, M610F007, which was previously described in literature as a preferred product formed after treatment of plants with DCA (see CA 6.2.1/2 and 6.2.1/3).

The deviating ratio of parent iprodione and its main metabolite RP30228 in carrot roots is probably partly due to the extraction with methanol. In addition, the different distribution of metabolites, with higher ratios of RP36115 and RP44247 can also be typical for roots where an uptake from soil could take place. The metabolite pattern in carrot tops, however, is very comparable with that of the older metabolism studies and therefore confirms the usual relation between the two compounds which is clear majority of parent in the beginning followed by gradual shift towards RP30228 in the course of the study.

In general, the new metabolism study is well in accordance with the existing ones. Furthermore, it adds data about the behavior in roots which were not known before. It completes the picture of the overall degradation reaction scheme and therefore explains the formerly partly inconsistent distribution of metabolites which can now be understood as complex system of equilibriums dependent on pH-values of crop, soil and solvents as well as on availability of possible reaction partners for conjugation.

Figure 6.2.1-3: General degradation scheme of iprodione in plant



CA 6.2.2 Poultry

In the framework of the original inclusion into Annex I according to Directive 91/414/EEC as well as during the re-evaluation according to Article 12 of Regulation (EC) No 396/2005 a metabolism study in laying hens (C022498) was peer-reviewed.

Table 6.2.2-1: Metabolism study in laying hen

Group	Species	Label position	No of animal	Application details		Sample details		Year	DocID
				Rate (mg/kg bw per d)	Duration (days)	Commodity	Time		
Laying poultry	Hens	^{14}C-phenyl	20	0.7	15	Eggs	During the dose period	1982	C022498
						Excreta	During the dose period		
						Tissues	2 hours, 3 days, 7 days		

In laying hens, the majority of radioactivity was observed in excreta and liver while in eggs, kidney, muscle and fat TRRs were lower. In eggs, RP36112, RP36115, RP44247 and RP 32490 (36.8% TRR) were the major metabolites identified. Parent Iprodione was present at lower levels. In liver and kidney, RP32490 and RP44247 were the major metabolites identified (22.4 % TRR and 26.7 % TRR in liver and 32.9 % TRR and 15.5 % TRR in kidney). Parent iprodione was present at lower levels. In muscle the predominant residue was RP32940 (74.2 % TRR). Iprodione and all other metabolites were <10 % TRR. In fat, parent iprodione accounted for 29.9% TRR, the metabolite RP32490 for 62.6 % TRR.

No further metabolism study was performed in poultry, since the metabolite patterns in livestock did not differ significantly. This is congruent with EFSA's 2013 statement that:
Metabolism in lactating ruminants and poultry was sufficiently investigated.

CA 6.2.3 Lactating ruminants

Metabolism studies in cow (C022496) and goat (C022497) were previously reviewed during the first Annex I-inclusion and recently confirmed in the 2013 EFSA review.

Table 6.2.3-1: Metabolism studies in ruminants

Group	Species	Label position	No of animal	Application details		Sample details		Year	DocID
				Rate (mg/kg bw per d)	Dura-tion (days)	Commo-dity	Time		
Lactating ruminants	Cow	^{14}C-phenyl	2 (a)	2	5	Milk	During the dose period	1981	C022496
						Urine and faeces	During the dose period		
						Tissues	At sacrifice (7 days after dosing)		
	Goat	^{14}C-phenyl	1	2	5	Milk	Twice daily	1982	C022497
						Urine and faeces	Twice daily		
						Tissues	At sacrifice 1982 (4 hours after dosing)		

In lactating cows the majority of the radioactivity was observed in the urine and faeces. TRR in organs, tissues and milk was lower. The 4-hydroxylated metabolite RP36114 and the non-hydroxylated metabolite RP32490 were the major metabolites of the residue (0.038 mg/kg, 24.7 % TRR and 0.036 mg/kg, 23.4 % TRR respectively). With the exception of liver, the total residue content of tissues was <0.05 mg/kg and therefore characterisation of these residues was not attempted

In goats the majority of radioactivity was observed in the urine 70.3 mg eq./kg. TRR in organs and tissues was lower. The metabolite RP32490 was the major metabolite in liver (1.11 mg/kg, 19.6 % TRR) whilst parent iprodione was identified at a lower level (13.4 % TRR). DCHPU was also detected in liver (0.505 mg/kg, 8.9 %) and in muscle (4.7 % TRR) and at lower levels in fat. RP36114 was detected in liver (1.3 % TRR) and at lower levels in muscle and fat. All other metabolites were present at <10 % TRR. In kidney, levels of iprodione were low (1.8 % TRR). The major metabolites identified were DCHPU (22.7 % TRR) and RP32490 (11.8 % TRR). In muscle and fat the major metabolite identified was RP 32490 (35.6 % TRR and 68 % TRR respectively). Parent iprodione was present at lower levels (1.4 % TRR and 7.6 % TRR respectively).

No further metabolism study was performed in ruminants, since the metabolite patterns in rodents (rats) and ruminants (goats) did not differ significantly. This is congruent with EFSA's 2013 statement that:
Metabolism in lactating ruminants and poultry was sufficiently investigated.

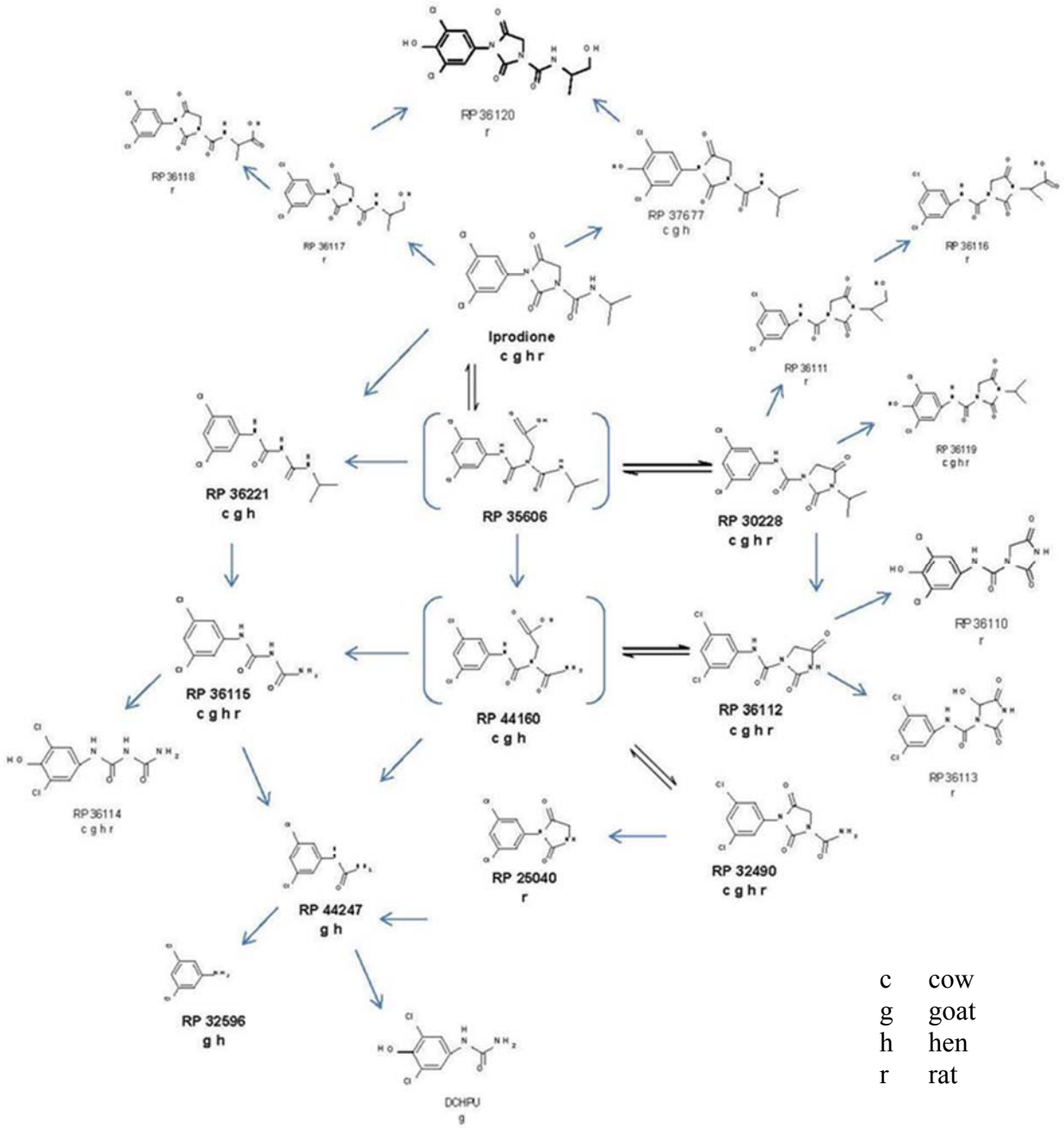
CA 6.2.4 Pigs

No metabolism study was performed in pigs, since the metabolite patterns in rodents (rats) and ruminants (goats) did not differ significantly. This is congruent with EFSA's 2013 statement that:
Metabolism in lactating ruminants and poultry was sufficiently investigated and findings can be extrapolated to pigs as well.

Overall Summary Livestock Metabolism

The general metabolic pathway in rodents and ruminants was found to be comparable. Ensuing from the degradation routes as explained for plant metabolism, oxidation reactions are observed mainly at the isopropyl group and/or at the phenyl ring leading to a number of hydroxylated and carboxylic derivatives. A complete picture including ruminant, poultry and rodent results is shown in Figure 6.2.4-1.

Figure 6.2.4-1: General degradation scheme of iprodione in animal



CA 6.2.5 Fish

According to Commission regulation 283/2013, metabolism studies in fish may be required where the plant protection product is used in crops whose parts or products, also after processing, are fed to fish and where residues in feed may occur from the intended applications.

The conditions under which such a study should be performed are further described in the Working document of the EU Commission SANCO/11187/2013, rev. 3 on the nature of pesticide residues in fish. The document specifies that the accumulation of compounds with low lipophilicity via the diet is known to be negligible and that fish metabolism studies are therefore required for active substances with a log P_{ow} equal or greater than 3 and an expected feed burden above 0.1 mg/kg DM.

In case of iprodione the log P_{ow} is 2.99 at pH 3 and 3.0 at pH 5 (see Review Report, 5036/VI/98-final 3. December 2002). In this dossier, carrot and lettuce are submitted which are not considered to serve as feed item for fish. Although the risk assessment for the consumer and the feed burden calculation for livestock in this dossier are based on the crops intended in future, no further study for fish was intended at this point in time because the final guideline in this subject is not yet published.

In addition, a valid bioaccumulation and depuration study in bluegill sunfish is available for iprodione (BASF DocID R014525, EU-accepted) which shows that a bioaccumulation of iprodione in aquatic organisms is unlikely (BCF: whole/ edible/non-edible: 46.8 / 34.8 / 70). Furthermore, the main compounds identified in edible portions were RP32490, parent iprodione, RP25040 and RP30228. In the inedible fraction RP36119 was found in addition. These findings are in full accordance with results from metabolism studies in livestock.

CA 6.3 Magnitude of residues trials in plants

CA 6.3.1 Carrot

Table 6.3.1-1: cGAP for the use of BAS 610 F in/on carrots

Crop	Maximum applied dose	Water volume	PHI	Application method	Application timing
Carrot	4 x 0.750 kg BAS 610 F/ha	200-800 L/ha	27	spray application	BBCH 13-49

PHI = pre-harvest interval

Table 6.3.1-2: GAP information of residue trials conducted in carrots in 1995-2011 in Northern Europe

Region	Country (No of trials) Year	Formu-lation	Application[0]				DALA[1]	DocID	EU accepted
			Method	Rate[0] (kg a.s./ha)	Spray conc. (kg a.s./hL)	No			
Northern EU	Netherlands (3) 1995	EXP01862F SC	spray appl.	0.700	0.09	4	27	C023294	yes
	Netherlands (5) 1996	EXP01862F SC	spray appl.	0.700	0.09	4	27	C023296	yes
	France (1) 1998	EXP01862F SC	spray appl.	0.730	0.22	3	26 40	C025546	no
	Germany (1) Netherlands (1) France (1) United Kingdom (1) 2010	BAS 610 06 F WG	spray appl.	0.750	0.15	4	0 21 ±2 28-±2 35-±2	2011/ 1120989	no
	Germany (1) Netherlands (1) France (1) United Kingdom (1) 2011	BAS 610 06 F WG	spray appl.	0.750	0.15	4	0 21 ±2 28-±2 35-±2	2013/ 1106641	no

0 Actual application rates varied by 10% at most
1 Days after last application

Table 6.3.1-3: GAP information of residue trials conducted in carrots in 1998-2011 in Southern Europe

Region	Country (No of trials) Year	Formulation	Application[0]				DALA[1]	DocID	EU accepted
			Method	Rate[0] (kg a.s./ha)	Spray conc. (kg a.s./hL)	No			
Southern EU	France (1) 1998	EXP01862F SC	spray appl.	0.750	0.12	3	26 40	C025546	no
	France (1) Greece (1) Italy (1) Spain (1) 2004	BAS 610 05 F SC	spray appl.	0.750	0.15	4	0 21 ±2 28-±2 35-±2	2004/ 7007471	no
	France (1) Greece (1) Italy (1) Spain (1) 2003	BAS 610 05 F SC	spray appl.	0.750	0.15	4	0 21 ±2 28-±2 35-±2	2005/ 1004980	no
	France (1) Greece (1) Italy (1) Spain (1) 2010	BAS 610 06 F WG	spray appl.	0.750	0.15	4	0 21 ±2 28-±2 35-±2	2011/ 1120989	no
	France (1) Greece (1) Italy (1) Spain (1) 2011	BAS 610 06 F WG	spray appl.	0.750	0.15	4	0 21 ±2 28-±2 35-±2	2013/ 1106641	no

0 Actual application rates varied by 10% at most
1 Days after last application

The studies not yet evaluated are summarized in the following chapter.

Report: CA 6.3.1/1
Baudet L., 1999b
Iprodione - Formulation EXP01862F (SC) North / France / 1998 - 1 decline study trial. South / France / 1998 - 1 decline study trial - Residues in carrot (root)
C025546
Guidelines: EEC 91/414
GLP: yes
(certified by Groupe Interministeriel des Produits Chimiques, France)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	EXP01862F (SC)
Lot/Batch #:	OP971126, 500 g/kg iprodione, nominal
Purity:	Not reported
CAS#:	36734-19-7
Development code:	Not reported
Spiking levels:	0.02 mg/kg

2. Test Commodity:

Crop:	Carrots
Type:	Root and tuber vegetables
Variety:	Major, Presto
Botanical name:	*Daucus carota*
Crop parts(s) or processed commodity:	Roots
Sample size:	Minimum 2 kg (12 plants)

B. STUDY DESIGN

1. Test procedure

During the 1998 growing season 2 trials in carrots were conducted in different representative growing areas in the North and South of France, to determine the residue level of iprodione in or on raw agricultural commodities (RAC). ECP01862F (500 g/L iprodione, SC) was applied three times at a rate of 0.780 kg iprodione/ha in spray volumes of 330-660 L/ha. Specimens of carrots were collected 26-29 days (BBCH 41-47); 40-41 days (BBCH 46-49) and in subplot 3R 55-56 days (BBCH 49) after the last application. Samples were stored frozen at or below -18°C for a maximum of 175 days until analysis.
The study contains more trials not meeting the intended GAP which are therefore not included in this dossier.

Table 6.3.1-4: Target application rates and timings for carrot

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
1998	2	3	F	EXP01862F (SC)	BAS 610 F	0.800	333-667	68-70 days 55-56 days 40 days before harvest

2. Description of analytical procedures

The specimens were analysed for iprodione (BAS 610 F) with BASF Method No. 144-97, quantifying iprodione with a LOQ of 0.02 mg/kg. Residues of iprodione are extracted by macerating with acetone. After addition of water and sodium chloride, a liquid-liquid partition with dichloromethane is performed. The extracts are purified using Sep-Pak plus Diol-cartridge (elution with a mixture of cyclohexane/toluene). The final determination of iprodione was performed by GC using an ELCD (electro conductivity detector).

Table 6.3.1-5: Summary of recoveries of iprodione and its metabolites in/on carrot

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 114-97		**BAS 610 F**		
Carrot root	0.02 / 0.04	6	92.5	12.6

II. RESULTS AND DISCUSSION

The residue results for the different trials are shown in Table 6.3.1-6 and Table 6.3.1-7.

In Northern Europe, residues of iprodione after 3 applications in carrot roots were found to be 0.17 mg/kg at 26 DALA to 0.19 mg/kg after 40 DALA.
In Southern Europe, residues of iprodione after 3 applications in carrot roots were 0.035 mg/kg at 26 DALA to 0.030 mg/kg after 40 DALA.

No residues at or above the LOQ (0.01 mg/kg) were found in any control sample.

III. CONCLUSION

At the intended PHI of 27 days, the residues of iprodione in carrot roots were 0.17 mg/kg in Northern and 0.03 mg/kg in Southern Europe.

Table 6.3.1-6: Residues of iprodione in carrots after two or three applications of EXP01862F (SC) in Northern Europe

Study details		Crop	Country	Formulation application rate (kg a.s./ha)[0)]	GS [2)] BBCH	DALA[1)]	Residues found (mg/kg)	
							Matrix	Iprodione
Study code:	98-750	Carrot	France	EXP01862F	47	26	Roots	0.17
DocID:	C025546			SC		40	Roots	0.19
Trial No:	98750RN1			3 x 0.800				
GLP:	Yes							
Year:	1998							

0 Variation of 10% at most
1 Days after last application
2 At last application

Table 6.3.1-7: Residues of iprodione in carrots after two or three applications of EXP01862F (SC) in Southern Europe

Study details		Crop	Country	Formulation application rate (kg a.s./ha)[0)]	GS [2)] BBCH	DALA[1)]	Residues found (mg/kg)	
							Matrix	Iprodione
Study code:	98-750	Carrot	France	EXP01862F	15-16	26	Roots	0.035
DocID:	C025546			SC		40	Roots	0.030
Trial No:	98750AV1			3 x 0.780				
GLP:	yes							
Year:	1998							

0 Variation of 10% at most
1 Days after last application
2 At last application

Report: CA 6.3.1/2
Jordan J., 2005b
Study on the residue behaviour of Iprodione in carrots after treatment with BAS 610 05F under field conditions in Southern France, Greece, Italy and Spain 2004
2004/7007471
Guidelines: EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7, EEC 1607/VI/97 rev. 2
10.06.1999
GLP: yes
(certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany Fed.Rep.)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 610 05 F (SC)
Lot/Batch #:	OP220391 & 1006, 500 g/L iprodione, nominal
Purity:	Not reported
CAS#:	36734-19-7
Development code:	Not reported
Spiking levels:	0.01, 0.10 mg/kg

2. Test Commodity:

Crop:	Carrots
Type:	Root and tuber vegetables
Variety:	Nantesa, Maestro, Bollero, Nando
Botanical name:	*Daucus carota*
Crop parts(s) or processed commodity:	Roots
Sample size:	1-2 kg (12 plants)

B. STUDY DESIGN

1. Test procedure

During the 2004 growing season 4 trials in carrots were conducted in different representative growing areas in the Southern EU, to determine the residue level of iprodione in or on raw agricultural commodities (RAC). BAS 610 05 F (500 g/L iprodione, SC) was applied four times at a rate equivalent to 0.750 kg iprodione/ha in spray volumes of 500 L/ha. The applications were performed 58±1 days before the anticipated harvest (1st application), 48±1 days before the anticipated harvest (2nd application), 38±1 days before the anticipated harvest (3rd application) and 28±1 days before the anticipated harvest (4rd application). Specimens of carrots were collected immediately after the last application (BBCH 43-49). Additional samples were taken 20-21 days (BBCH 45-51), 27-28 days (BBCH 47-51) and 34-35 days (BBCH 49-51) after the last application. Samples were stored frozen at or below -18°C for a maximum of 150 days until analysis.

Table 6.3.1-8: Target application rates and timings for carrot

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	4	F	BAS 610 05 F (SC)	BAS 610 F	0.750	500	58±1 days 48±1 days 38±1 days 28±1 days before harvest

2. Description of analytical procedures

The specimens were analysed for parent iprodione (BAS 610 F) with BASF Method No. 543/0, quantifying iprodione with a LOQ of 0.01 mg/kg. Residues were extracted from carrot matrices with a mixture of acetone, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned against cyclohexane. The final determination was performed by HPLC-MS/MS.

Table 6.3.1-9: Summary of recoveries of iprodione and its metabolites in/on carrot

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF Method No 543/0		**BAS 610 F**		
whole plant, roots	0.01-0.1	4	84.2	11.7

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.1-10 detailed residue levels are shown in Table 6.3.1-11.
Residues of iprodione declined in carrot from 5.64-38.65 mg/kg at 0 DALA in whole plant commodities to 0.11-0.30 mg/kg after 27±1 DALA in roots.
No residues at or above the LOQ (0.01 mg/kg) were found in any control sample.

Table 6.3.1-10: Summary of residues in carrot

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Matrix	Range of iprodione residues (mg/kg)
Southern EU	2004	0	43-49	Wh. plant w/o root	5.64-38.65
		20-21	45-51	Roots	0.10-0.34
		27-28	47-51	Roots	0.11-0.30
		34-35	49-51	Roots	0.08-0.22

1 Days after last application
2 At harvest

III. CONCLUSION

Residues of iprodione declined in carrot commodities during the harvesting period. In the Southern EU residues of iprodione declined in carrot from 5.64-38.65 mg/kg at 0 DALA in whole plant commodities to 0.08-0.22 mg/kg after 35±1 DALA in roots.
At the intended PHI of 27 days, the residues of iprodione in carrot roots were 0.11-0.30 mg/kg.

Table 6.3.1-11: Residues of iprodione in carrots after four applications of BAS 610 05 F (SC) in Southern Europe

Study details		Crop	Country	Formulation application rate (kg a.s./ha)[0)]	GS [2)] BBCH	DALA[1)]	Residues found (mg/kg)	
							Matrix	Iprodione
Study code:	170830	Carrot	Spain	BAS 610 05 F	43	0	Wh. plant*	38.65
DocID:	2004/7007471			SC		21	Roots	0.10
Trial No:	ALO/33/04			4 x 0.750		27	Roots	0.11
GLP:	Yes					35	Roots	0.08
Year:	2004							
Study code:	170830	Carrot	France	BAS 610 05 F	45	0	Wh. plant*	13.22
DocID:	2004/7007471			SC		21	Roots	0.15
Trial No:	FTL/22/04			4 x 0.750		28	Roots	0.16
GLP:	Yes					35	Roots	0.18
Year:	2004							
Study code:	170830	Carrot	Greece	BAS 610 05 F	43	0	Wh. plant*	19.46
DocID:	2004/7007471			SC		20	Roots	0.38
Trial No:	GRE/23/04			4 x 0.750		27	Roots	0.30
GLP:	Yes					34	Roots	0.22
Year:	2004							
Study code:	170830	Carrot	Italy	BAS 610 05 F	49	0	Wh. plant*	5.64
DocID:	2004/7007471			SC		21	Roots	0.24
Trial No:	ITA/25/04			4 x 0.750		28	Roots	0.15
GLP:	Yes					35	Roots	0.13
Year:	2004							

0 Variation of 10% at most
1 Days after last application
2 At last application
* Whole plants without roots

Report: CA 6.3.1/3
Schulz H., 2005b
Study on the residue behaviour of Iprodione in carrots after treatment with BAS 610 05 F under field conditions in Southern France, Italy and Spain 2003
2005/1004980
Guidelines: EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 610 05 F (SC)
Lot/Batch #: OP220391, 500 g/kg iprodione, nominal
Purity: Not reported
CAS#: 36734-19-7
Development code: Not reported
Spiking levels: 0.01, 0.10 mg/kg

2. Test Commodity:

Crop: Carrots
Type: Root and tuber vegetables
Variety: Tino F1, Puma, Nandor
Botanical name: *Daucus carota*
Crop parts(s) or processed commodity: Roots
Sample size: 1-2 kg (12 plants)

B. STUDY DESIGN

1. Test procedure

During the 2003 growing season 4 trials in carrots were conducted in different representative growing areas in the Southern EU, to determine the residue level of iprodione in or on raw Agricultural Commodities (RAC). BAS 610 05 F (500 g/kg iprodione, SC) was applied four times at a rate equivalent to 0.750 kg iprodione/ha in spray volumes of 500 L/ha. The applications were performed 58±1 days before the anticipated harvest (1st application), 48±1 days before the anticipated harvest (2nd application), 38±1 days before the anticipated harvest (3rd application) and 28±1 days before the anticipated harvest (4rd application). Specimens of carrots were collected immediately after the last application (BBCH 41-47). Additional samplings were taken 21 days (BBCH 41-48), 28 days (BBCH 49) and 35 days (BBCH 49) after the last application. Samples were stored frozen at or below -18°C for a maximum of 641 days until analysis.

Table 6.3.1-12: Target application rates and timings for carrot

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	4	4	F	BAS 610 05 F (SC)	BAS 610 F	0.750	500	58±1 days 48±1 days 38±1 days 28±1 days before harvest

2. Description of analytical procedures

The specimens were analysed for iprodione (BAS 610 F) with BASF Method No. 543/0, quantifying iprodione with a LOQ of 0.01 mg/kg. Residues were extracted from carrot matrices with a mixture of acetone, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned against cyclohexane. The final determination was performed by HPLC-MS/MS.

Table 6.3.1-13: Summary of recoveries of iprodione and its metabolites in/on carrot

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF Method No 543/0		**BAS 610 F**		
whole plant	0.01-0.1	4	86.7	7.7
root				

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.1-14, detailed residue levels are shown in Table 6.3.1-15.
Residues of iprodione declined in carrot from 8.08-18.94 mg/kg at 0 DALA in whole plant commodities to 0.04-0.46 mg/kg after 28 DALA in roots.
No residues at or above the LOQ (0.01 mg/kg) were found in any control sample.

Table 6.3.1-14: Summary of residues in carrot

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Matrix	Range of iprodione residues (mg/kg)
Southern EU	2003	0	41-47	Wh. plant w/o root	8.08-18.94
		21	47-48	Roots	0.05-0.42
		28	49	Roots	0.04-0.46
		35	49	Roots	0.03-0.39

1 Days after last application
2 At harvest

III. CONCLUSION

Residues of iprodione declined in carrot commodities during the harvesting period. In the Southern EU residues of iprodione declined in carrot from 8.08-18.94 mg/kg at 0 DALA in whole plant commodities to 0.03-0.39 mg/kg after 28 DALA in roots.
At the intended PHI of 27 days, the residues of iprodione in carrot roots were 0.04-0.46 mg/kg.

Table 6.3.1-15: Residues of iprodione in carrots after four applications of BAS 610 05 F (SC) in Southern Europe

Study details		Crop	Country	Formulation application rate (kg a.s./ha)[0)]	GS [2)] BBCH	DALA[1)]	Residues found (mg/kg)	
							Matrix	Iprodione
Study code:	170827	Carrot	Spain	BAS 610 05 F	41	0	Wh. plant*	11.68
DocID:	2005/1004980			SC		21	Roots	0.42
Trial No:	ALO/31/03			4 x 0.750		28	Roots	0.46
GLP:	Yes					35	Roots	0.39
Year:	2003							
Study code:	170827	Carrot	France	BAS 610 05 F	41	0	Wh. plant*	9.12
DocID:	2005/1004980			SC		21	Roots	0.40
Trial No:	ALO/32/03			4 x 0.750		28	roots	0.40
GLP:	Yes					35	roots	0.37
Year:	2003							
Study code:	170827	Carrot	Greece	BAS 610 05 F	43	0	Wh. plant*	18.94
DocID:	2005/1004980			SC		21	Roots	0.05
Trial No:	FBD/20/03			4 x 0.750		28	roots	0.04
GLP:	Yes					35	roots	0.03
Year:	2003							
Study code:	170827	Carrot	Italy	BAS 610 05 F	47	0	Wh. plant*	8.08
DocID:	2005/1004980			SC		21	Roots	0.31
Trial No:	ITA/21/03			4 x 0.750		28	roots	0.23
GLP:	Yes					35	roots	0.23
Year:	2003							

0 Variation of 10% at most
1 Days after last application
2 At last application
* Whole plants without roots

Report: CA 6.3.1/4
Moreno S., 2011b
Study on the residue behaviour of BAS 610 F in carrot, after treatment with BAS 610 06 F under field conditions in North and South Europe, season 2010
2011/1120989

Guidelines: EEC 87/18 (No. L 15/29) 1986, International guidelines for distribution and pesticides application AEPLA FAO 1985, EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5 Appendix B

GLP: yes
(certified by ENAC, Entidad Nacional de Acreditación, Madrid Spain)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 610 06 F (WG)
Lot/Batch #: 08-160018, 750 g/kg iprodione, nominal
Purity: Not reported
CAS#: 36734-19-7
Development code: Not reported
Spiking levels: 0.01-50.0 mg/kg

2. Test Commodity:

Crop: Carrots
Type: Root and tuber vegetables
Variety: Maestro, Maxi, Karotan, Nairobi, Tempo F1, Nandor, Nipo
Botanical name: *Daucus carota*
Crop parts(s) or processed commodity: Roots, tops
Sample size: 12 plants

B. STUDY DESIGN

1. Test procedure

During the 2010 growing season 8 trials in carrots were conducted in different representative growing areas in the Northern and Southern EU, to determine the residue level of iprodione and its metabolites RP30228, RP32490 and RP32596 in or on raw agricultural commodities (RAC).
The carrot root samples were also analysed for the recently discovered metabolite M610F007 in a separate study. The analytical details are described in CA 6.3.1/6, the results thereof are also included in this summary.
BAS 610 06 F (750 g/kg iprodione, WG) was applied four times at a rate equivalent to 0.750 kg iprodione/ha in spray volumes of 500 L/ha. The applications were performed 57±1 days before the anticipated harvest (1st appl., BBCH 13/14-45), 47±1 days before the anticipated harvest (2nd appli., BBCH 41-46), 37±1 days before the anticipated harvest (3rd appli., BBCH 41-47) and 27±1 days before the anticipated harvest (4rd appli., BBCH 43-49). Specimens of carrots were collected immediately after the last application (BBCH 43-49). Additional samples were taken 20-22 days (BBCH 45-49), 26-28 days (BBCH 47-49) and 35-45 days (BBCH 49) after the last application. Samples were stored frozen at or below -18°C for a maximum of 393 days until analysis.

Table 6.3.1-16: Target application rates and timings for carrot

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
2010	8	4	F	BAS 610 06 F (WG)	BAS 610 F	0.750	500	57±1 days 47±1 days 37±1 days 27±1 days before harvest

2. Description of analytical procedures

The specimens were analysed for iprodione (BAS 610 F) and its metabolites RP 30228, RP 32490 and RP 32596 with BASF method No L0180/01, quantifying each relevant analyte with an LOQ of 0.01 mg/kg. Residues were extracted from carrot matrices with a mixture of acetonitrile and water. An aliquot of the extract was centrifuged and measured directly. The final determination of all analytes was performed by UPLC-MS/MS.

Table 6.3.1-17: Summary of recoveries of iprodione and its metabolites in/on carrot

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No L0180/01		**BAS 610 F**			**RP 32490 (Reg. No 5079628)**		
whole plant, with roots, tops	0.01-50	27	99.8	6.3	27	99.4	5.1
BASF method No L0180/01		**RP 30228 (Reg. No 5079647)**			**RP 32596 (Reg. No 85831)**		
whole plant, with roots, tops	0.01-50	27	99.3	5.5	27	95.6	6.5

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.1-18, detailed residue levels are shown in Table 6.3.1-19 and Table 6.3.1-20.

Residues of iprodione declined in carrot from 4.48-35.28 mg/kg at 0 DALA in whole plant commodities to 0.09-0.35 mg/kg after 27±1 DALA in roots. In the top of the plants, residues of iprodione ranged between 1.54-8.88 mg/kg at 27±1 DALA.

Of the metabolites, only residues of RP 30228 ranged between <0.01-0.04 mg/kg were found in carrot root commodities at 27±1 DALA; all other metabolites were <0.01 mg/kg (LOQ).
In the top of the plants residues of the metabolites ranged between 0.01-0.09 mg/kg for RP 32490, 0.06-0.20 mg/kg for RP 30228 and <0.01-0.02 mg/kg for RP 32596 at 27±1 DALA.
The glutamic acid conjugate M610F007 was found in all carrot root samples between 0.04 and 0.35 mg/kg.

No residues at or above the LOQ (0.01 mg/kg) were found in any control sample.

Table 6.3.1-18: Summary of residues in carrot

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Matrix	Range of residues (mg/kg)				
					iprodione	RP 30228 [3)]	RP 32490 [4)]	RP 32596	M610F007
					as parent equivalents				
Northern EU	2010	0	35-45	Wh. plant*	4.48-8.29	0.01-0.05	<0.01-0.02	<0.01	-
		20-22	41-46	Roots	0.07-0.18	<0.01-0.02	<0.01	<0.01	0.07-0.32
		26-28	43-47		0.09-0.20	<0.01-0.01	<0.01	<0.01	0.08-0.35
		35	45-48		0.05-0.15	<0.01-0.01	<0.01	<0.01	0.04-0.29
		20-22	41-46	Tops	2.16-11.50	0.06-0.21	0.02-0.10	<0.01-0.01	-
		26-28	43-47		1.54-6.42	0.08-0.20	0.01-0.08	<0.01-0.02	-
		35	45-48		0.80-6.48	0.08-0.33	<0.01-0.11	<0.01-0.01	-
Southern EU	2010	0	13-43	Wh. plant*	7.26-35.28	0.03-0.12	0.02-0.08	<0.01	
		20-22	41-43	Roots	0.11-0.34	0.01-0.03	<0.01	<0.01	0.04-0.12
		26-28	41-45		0.12-0.35	0.02-0.04	<0.01	<0.01	0.05-0.11
		35	43-45		0.09-0.36	0.01-0.04	<0.01	<0.01	0.08-0.25
		20-22	41-43	Tops	2.06-9.62	0.04-0.20	0.03-0.10	<0.01	
		26-28	41-45		2.12-8.88	0.06-0.17	0.02-0.09	<0.01	
		35	43-45		1.54-8.36	0.04-0.19	0.02-0.07	<0.01	

1 Days after last application
2 At harvest
3 RP 030228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4 RP 032490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
* Whole plant with roots

III. CONCLUSION

Residues of iprodione and its metabolites RP 30228 declined in carrot commodities during the harvesting period.
At the intended PHI of 27 days, the residues of iprodione in carrot roots were found up to 0.20 mg/kg in Northern and to 0.35 mg/kg in Southern Europe. RP 30228 was found as main metabolite with a maximum of 0.04 mg/kg at the intended PHI. Metabolite RP 32490 was not found in carrot root, only in tops up to 0.11 mg/kg.
Metabolite RP 32596 was not found above LOQ in edible plant parts. In two out of 8 trials it was detected in carrot tops up to 0.02 mg/kg
The glutamic acid derivative M610F007 was observed in roots up to 0.35 mg/kg.

Table 6.3.1-19: Residues of iprodione in carrots after four applications of BAS 610 06 F (WG) in Northern Europe

Study details		Crop	Country	Formulation application Rate (kg a.s./ha)[0)]	GS[2] BBCH	DA-LA[1]	Matrix	Residues (mg/kg)				
								iprodione	RP 30228 [3)]	RP 32490 [4)]	RP 32596	M610 F007
								as parent equivalents				
Study code:	384192	Carrot	Germany	BAS	48	0	Wh. plant*	8.29	0.02	0.01	<0.01	-
DocID:	2011/1120989			610 06 F		22	roots	0.18	<0.01	<0.01	<0.01	0.28
Trial No:	L100668			WG		22	Tops	11.50	0.08	0.09	<0.01	-
GLP:	Yes			4 x 0.750		28	roots	0.20	<0.01	<0.01	<0.01	0.24
Year:	2010					28	Tops	6.42	0.08	0.07	<0.01	-
						35	Roots	0.15	<0.01	<0.01	<0.01	0.29
						35	Tops	6.25	0.10	0.08	<0.01	-
Study code:	384192	Carrot	The	BAS 610 06 F	45	0	Wh. plant*	4.48	0.05	<0.01	<0.01	-
DocID:	2011/1120989		Nether-	WG		20	roots	0.14	0.02	<0.01	<0.01	0.07
Trial No:	L100238		lands	4 x 0.750		20	Tops	2.16	0.21	0.02	<0.01	-
GLP:	Yes					28	roots	0.12	0.01	<0.01	<0.01	0.08
Year:	2010					28	Tops	1.54	0.16	0.01	<0.01	-
						35	roots	0.12	0.01	<0.01	<0.01	0.04
						35	Tops	0.80	0.08	<0.01	<0.01	-
Study code:	384192	Carrot	France	BAS 610 06 F	48	0	Wh. plant*	5.84	0.03	<0.01	<0.01	-
DocID:	2011/1120989			WG		22	roots	0.07	<0.01	<0.01	<0.01	0.32
Trial No:	L100239			4 x 0.750		22	Tops	3.28	0.06	0.02	<0.01	--
GLP:	Yes					26	roots	0.09	<0.01	<0.01	<0.01	0.32
Year:	2010					26	Tops	4.04	0.12	0.03	0.02	-
						35	roots	0.05	<0.01	<0.01	<0.01	0.26
						35	Tops	2.38	0.10	0.02	0.01	
Study code:	384192	Carrot	United	BAS 610 06 F	47	0	Wh. plant*	5.35	0.01	0.02	<0.01	
DocID:	2011/1120989		Kingdom	WG		21	roots	0.09	<0.01	<0.01	<0.01	0.14
Trial No:	L100240			4 x 0.750		21	Tops	4.24	0.20	0.10	0.01	-
GLP:	Yes					28	roots	0.09	<0.01	<0.01	<0.01	0.35
Year:	2010					28	Tops	5.60	0.20	0.08	0.01	-
						35	roots	0.09	<0.01	<0.01	<0.01	0.19
						35	Tops	6.48	0.33	0.11	<0.01	-

0 Variation of 10% at most
1 Days after last application
2 At last application
3 RP 030228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4 RP 032490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
* Whole plants with roots

Table 6.3.1-20: Residues of iprodione in carrots after four applications of BAS 610 06 F (WG) in Southern Europe

Study details		Crop	Country	Formulation application Rate (kg a.s./ha)[0)]	GS[2] BBCH	DA-LA[1]	Matrix	Residues (mg/kg)				
								iprodione	RP 30228 [3)]	RP 32490 [4)]	RP 32596	M610 F007
								as parent equivalents				
Study code	384192	Carrot	France	BAS 610 06 F	44	0	W. plant*	8.08	0.03	0.02	<0.01	-
DocID:	2011/1120989			WG		21	roots	0.26	0.01	<0.01	<0.01	0.12
Trial No:	L100241			4 x 0.750		21	tops	4.84	0.05	0.08	<0.01	-
GLP:	Yes					27	roots	0.31	0.02	<0.01	<0.01	0.11
Year:	2010					27	tops	3.92	0.08	0.07	<0.01	-
						35	roots	0.14	0.01	<0.01	<0.01	0.25
						35	tops	2.32	0.06	0.04	<0.01	-
Study code:	384192	Carrot	Greece	BAS 610 06 F	44	0	W. plant*	35.28	0.10	0.08	<0.01	-
DocID:	2011/1120989			WG		21	roots	0.34	0.02	<0.01	<0.01	0.04
Trial No:	L100242			4 x 0.750		21	tops	9.62	0.17	0.10	<0.01	-
GLP:	Yes					27	roots	0.27	0.02	<0.01	<0.01	0.05
Year:	2010					27	tops	8.88	0.17	0.09	<0.01	-
						35	roots	0.33	0.03	<0.01	<0.01	0.08
						35	tops	8.36	0.19	0.07	<0.01	-
Study code:	384192	Carrot	Italy	BAS 610 06 F	43-45	0	W. plant*	8.19	0.12	0.02	<0.01	-
DocID:	2011/1120989			WG		21	roots	0.29	0.03	<0.01	<0.01	-
Trial No:	L100243			4 x 0.750		21	tops	5.54	0.20	0.05	<0.01	-
GLP:	Yes					27	roots	0.35	0.04	<0.01	<0.01	-
Year:	2010					27	tops	5.43	0.16	0.04	<0.01	-
						35	roots	0.36	0.04	<0.01	<0.01	-
						35	tops	5.04	0.18	0.04	<0.01	-
Study code:	384192	Carrot	Spain	BAS 610 06 F	43	0	W. plant*	7.26	0.03	0.02	<0.01	-
DocID:	2011/1120989			WG		20	roots	0.11	0.01	<0.01	<0.01	-
Trial No:	L100244			4 x 0.750		20	tops	2.06	0.04	0.03	<0.01	-
GLP:	Yes					27	roots	0.12	0.02	<0.01	<0.01	-
Year:	2010					27	tops	2.12	0.06	0.02	<0.01	-
						34	roots	0.09	0.01	<0.01	<0.01	-
						34	tops	1.54	0.04	0.02	<0.01	-

0 Variation of 10% at most
1 Days after last application
2 At last application
3 RP 030228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4 RP 032490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
* Whole plants with roots

Report: CA 6.3.1/5
Perny A., 2013b
Study on the residue behaviour of BAS 610 F (Iprodione) after treatment with BAS 610 06 F in carrots under field conditions in Northern and Southern Europe, 2011
2013/1106641

Guidelines: EEC 1607/VI/97 rev. 2 10.06.1999, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 7029/VI/95 rev. 5 Appendix B, EEC 7525/VI/95 rev. 7

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

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 610 06 F (WG)
Lot/Batch #: 09-160007, 750 g/kg iprodione, nominal
Purity: Not reported
CAS#: 36734-19-7
Development code: Not reported
Spiking levels: 0.01, 0.10, 1.0, 10.0 mg/kg

2. Test Commodity:

Crop: Carrots
Type: Root and tuber vegetables
Variety: Komarno F1, Bolero, Newark, Entopio, Ceres, Evora
Botanical name: *Daucus carota*
Crop parts(s) or processed commodity: Roots, tops
Sample size: 1-2 kg (12 plants)

B. STUDY DESIGN

1. Test procedure

During the 2011 growing season 8 trials in carrots were conducted in different representative growing areas in the Northern and Southern EU, to determine the residue level of iprodione and its metabolites RP 30228, RP 32490, RP 32596 and RP 37176 in or on raw agricultural commodities (RAC). The carrot root samples were also analysed for the recently discovered metabolite M610F007 in a separate study. The analytical details are described in CA 6.3.1/6, the results thereof are also included in this summary.
BAS 610 06 F (750 g/kg iprodione, WG) was applied four times at a rate equivalent to 0.750 kg iprodione/ha in spray volumes of 500 L/ha. The applications were performed 56-62 days before the anticipated harvest (1st appl., BBCH 13-45), 45-50 days before the anticipated harvest (2nd appl., BBCH 15-45), 35-40 days before the anticipated harvest (3rd appl., BBCH 19-47) and 26-29 days before the anticipated harvest (4rd appl., BBCH 41-47). Specimens of carrots were collected immediately after the last application (BBCH 41-47). Additional samples were taken 20-22 days (BBCH 45-49), 26-28 days (BBCH 47-49) and 34-35 days (BBCH 49) after the last application. Samples were stored frozen at or below -18°C for a maximum of 271 days until analysis.

Table 6.3.1-21: Target application rates and timings for carrot

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
2011	8	4	F	BAS 610 06 F (WG)	BAS 610 F	0.750	500	57±1 days 47±1 days 37±1 days 27±1 days before harvest

2. Description of analytical procedures

The specimens were analysed for iprodione (BAS 610 F) and its metabolites RP 30228, RP 32490, RP 32596 and RP 37176 with BASF method No L0180/01, quantifying each relevant analyte with an LOQ of 0.01 mg/kg. Residues were extracted from carrot matrices with a mixture of acetonitrile and water. An aliquot of the extract was centrifuged and measured directly. The final determination of all analytes was performed by UPLC-MS/MS.

Table 6.3.1-22: Summary of recoveries of iprodione and its metabolites in/on carrot

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No L0180/01		**BAS 610 F**			**RP 30228 (Reg. No 5079647)**		
whole plant*	0.01-10	6	101.0	8.7	9	100.0	7.8
roots	0.01-10	6	103.0	6.3	6	100.0	7.5
top	0.01-10	9	96.5	5.9	9	92.6	4.3
overall	*0.01-10*	*21*	*100.0*	*7.2*	*24*	*97.2*	*7.4*
BASF method No L0180/01		**RP 32490 (Reg. No 5079628)**			**RP 37176 (Reg. No. 5079612)**		
whole plant*	0.01, 0.10	4	98.9	8.7	4	103.0	3.3
roots	0.01, 0.10	4	97.6	15	4	96.7	7.6
top	0.01-10	9	93.6	3.6	9	98.6	5.1
overall	*0.01-10*	*17*	*95.8*	*8.5*	*17*	*99.2*	*5.6*
BASF method No L0180/01		**RP 32596 (Reg. No 85831)**					
whole plant*	0.01-10	6	91.5	10.0			
roots	0.01-10	6	101.0	5.1			
top	0.01-10	9	83.4	11.0			
overall	*0.01-10*	*21*	*90.7*	*12.0*			

* with roots

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.1-23, detailed residue levels are shown in Table 6.3.1-24 and Table 6.3.1-25.
Residues of iprodione declined in carrot from 2.80-32 mg/kg at 0 DALA in whole plant commodities to 0.06-0.45 mg/kg after 27±1 DALA in roots. In the top of the plants, residues ranged between 0.98-21.00 mg/kg for iprodione at 26-28 DALA,
Of the metabolites, only residues of RP 30228 ranged between 0.01-0.05 mg/kg in carrot root commodities at 27±1 DALA; all other metabolites were <0.01 mg/kg (LOQ).
Regarding residue levels in carrot top, all results can be taken into account with the exception of the values obtained from the UK trial where an erroneous additional application of an iprodione containing product was performed between the 3rd and 4th regular treatment. Both the ratio between parent iprodione and RP30228 and especially the amount of RP32596 at the end of the study deviate considerably from the results of the other trials and should therefore not be considered for any evaluation.
In Table 6.3.1-23 the residue ranges in carrot tops are shown without the UK results which are given in brackets.
No residues at or above the LOQ (0.01 mg/kg) were found in any control sample.

Table 6.3.1-23: Summary of residues in carrot

Region	Year	DALA [1]	Growth stage[2] (BBCH)	Matrix	Range of residues (mg/kg)					
					iprodione	RP 30228 [3]	RP 32490 [4]	RP 32596	RP 37176	M610 F007
					as parent equivalents					
N-EU	2011	0	41-47	Wh. pl.*	2.80-10.8	0.04-0.05	<0.01-0.01	<0.01	<0.01	-
		20-22	45-49	Roots	0.07-0.16	<0.01	<0.01	<0.01	<0.01	0.05-0.46
		26-29	47-49		0.06-0.08	<0.01	<0.01	<0.01	<0.01	0.05-0.38
		34-35	49		0.03-0.08	<0.01	<0.01	<0.01	<0.01	0.04-0.25
		20-22	45-49	Tops	0.88-3.12 (21.60) [5]	0.05-0.09 (1.01) [5]	<0.01-0.05 (0.07) [5]	<0.01 (0.03) [5]	<0.01	-
		26-29	47-49		0.98-2.28 (16.48) [5]	0.06-0.12 (1.09) [5]	<0.01-0.03 (0.06) [5]	<0.01 (0.03) [5]	<0.01 (0.01) [5]	-
		34-35	49		0.67-2.06 (6.82) [5]	0.06 (3.83) [5]	<0.01-0.02 (0.08) [5]	<0.01 (0.21) [5]	<0.01 (0.02) [5]	-
S-EU	2011	0	42-47	Wh. pl.*	6.10-32.20	0.04-0.08	<0.01-0.03	<0.01-0.01	<0.01-0.01	-
		20-22	47-49	Roots	0.13-0.46	0.01-0.05	<0.01	<0.01	<0.01	0.01-0.11
		26-28	48-49		0.11-0.45	0.01-0.05	<0.01	<0.01	<0.01	0.01-0.16
		35	49		0.09-0.46	0.01-0.09	<0.01	<0.01	<0.01	0.01-0.23
		20-22	47-49	Tops	2.30-33.0	0.08-0.31	0.02-0.50	<0.01	<0.01	-
		26-28	48-49		1.20-21.00	0.02-0.31	0.01-0.33	<0.01	<0.01	-
		35	49		1.40-12.00	0.06-0.85	<0.01-0.28	<0.01-0.01	<0.01	-

1 Days after last application
2 At harvest
3 RP 030228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4 RP 032490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
5 These results should not be taken into account in any evaluation because they are the outcome of an erroneous additional application of iprodione.
* Whole plant without roots

t

III. CONCLUSION

Residues of iprodione and its metabolites RP 030228, RP 032490 and RP 032596 declined in carrot roots during the harvesting period.
At the intended PHI of 27 days, the residues of iprodione in carrot roots were found up to 0.08 mg/kg in Northern and to 0.45 mg/kg in Southern Europe.
RP 30228 was found as main metabolite with a maximum of 0.05 mg/kg at the intended PHI. Metabolites RP 32490, RP 32596 and RP 37176 were not found above LOQ in edible plant parts.
The glutamic acid derivative M610F007 is generally observed in roots only.

Table 6.3.1-24: Residues of iprodione in carrots after four applications of BAS 610 06 F (WG) in Northern Europe

Study details		Crop	Country	Formulation application Rate (kg a.s./ha)[0)]	GS[2] BBCH	DA-LA[1]	Matrix	Residues (mg/kg)					
								iprodione	RP 30228 [3)]	RP 32490 [4)]	RP 32596	RP 37176	M610 F007
								as parent equivalents					
Study code	384194	Carrot	The	BAS 610 06 F	45	0	W. plant*	11.0	0.05	<0.01	<0.01	<0.01	-
DocID:	2013/1106641		Nether-	WG		21	Roots	0.16	<0.01	<0.01	<0.01	<0.01	0.10
Trial No:	L110383		lands	4 x 0.750		21	Tops	0.88	0.05	<0.01	<0.01	<0.01	-
GLP:	Yes					28	Roots	0.08	<0.01	<0.01	<0.01	<0.01	0.11
Year:	2011					28	Tops	0.98	0.06	<0.01	<0.01	<0.01	-
						35	Roots	0.08	<0.01	<0.01	<0.01	<0.01	0.06
						35	Tops	0.67	0.06	<0.01	<0.01	<0.01	-
Study code:	384194	Carrot	Germany	BAS 610 06 F	41-43	0	W. plant*	10.8	0.04	0.01	<0.01	<0.01	-
DocID:	2013/1106641			WG		21	Roots	0.12	<0.01	<0.01	<0.01	<0.01	0.05
Trial No:	L110384			4 x 0.750		21	Tops	3.12	0.09	0.05	<0.01	<0.01	-
GLP:	Yes					26	Roots	0.07	<0.01	<0.01	<0.01	<0.01	0.05
Year:	2011					26	Tops	2.28	0.12	0.03	<0.01	<0.01	-
						34	Roots	0.07	<0.01	<0.01	<0.01	<0.01	0.04
						34	Tops	2.06	0.06	0.03	<0.01	<0.01	-
Study code:	384194	Carrot	France	BAS 610 06 F	45-47	0	W. plant*	2.80	0.04	<0.01	<0.01	<0.01	-
DocID:	2013/1106641			WG		22	Roots	0.07	<0.01	<0.01	<0.01	<0.01	0.20
Trial No:	L110385			4 x 0.750		22	Tops	2.03	0.09	0.02	<0.01	<0.01	-
GLP:	Yes					29	Roots	0.08	<0.01	<0.01	<0.01	<0.01	0.19
Year:	2011					29	Tops	1.76	0.09	0.01	<0.01	<0.01	-
						35	Roots	0.08	<0.01	<0.01	<0.01	<0.01	0.16
						35	Tops	1.61	0.06	0.02	<0.01	<0.01	-
Study code:	384194	Carrot	United	BAS 610 06 F	43-45	0	W. plant*	4.44	0.04	<0.01	<0.01	<0.01	-
DocID:	2013/1106641		Kingdom	WG		20	Roots	0.08	<0.01	<0.01	<0.01	<0.01	0.46
Trial No:	L110386			4 x 0.750 #		20	Tops	21.60	1.01	0.07	0.03	<0.01	-
GLP:	Yes					27	Roots	0.06	<0.01	<0.01	<0.01	<0.01	0.38
Year:	2011					27	Tops	16.48	1.09	0.06	0.03	0.01	-
						35	Roots	0.03	<0.01	<0.01	<0.01	<0.01	0.25
						35	Tops	6.82	3.83	0.08	0.21	0.02	-

0 Variation of 10% at most
1 Days after last application
2 At last application
3 RP 030228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4 RP 032490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
erroneously, a treatment with COMPASS (BAS 613 01 F) was done, resulting in an additional rate of 225 g iprodione applied between 3rd and 4th regular treatment. The results in tops should not be taken into account in any evaluation.
* Whole plants with roots

Table 6.3.1-25: Residues of iprodione in carrots after four applications of BAS 610 06 F (WG) in Southern Europe

Study details		Crop	Country	Formulation application Rate (kg a.s./ha)[0)]	GS[2] BBCH	DA-LA[1]	Matrix	Residues (mg/kg)					
								iprodione	RP 30228 [3)]	RP 32490 [4)]	RP 32596	RP 37176	M610 F007
								as parent equivalents					
Study code	384194	Carrot	France	BAS 610 06 F	45	0	W. plant*	10.00	0.06	<0.01	<0.01	0.01	-
DocID:	2013/1106641			WG		21	Roots	0.17	0.02	<0.01	<0.01	<0.01	0.11
Trial No:	L110387			4 x 0.750		21	Tops	2.36	0.10	0.02	<0.01	<0.01	-
GLP:	Yes					27	Roots	0.15	0.02	<0.01	<0.01	<0.01	0.08
Year:	2011					27	Tops	1.16	0.08	0.01	<0.01	<0.01	-
						35	Roots	0.11	0.02	<0.01	<0.01	<0.01	0.06
						35	Tops	1.42	0.07	0.02	<0.01	<0.01	-
Study code:	384194	Carrot	Greece	BAS 610 06 F	45-46	0	W. plant*	8.40	0.04	<0.01	<0.01	<0.01	-
DocID:	2013/1106641			WG		21	Roots	0.13	0.01	<0.01	<0.01	<0.01	0.01
Trial No:	L110388			4 x 0.750		21	Tops	3.31	0.19	0.05	<0.01	<0.01	-
GLP:	Yes					26	Roots	0.11	0.01	<0.01	<0.01	<0.01	0.01
Year:	2011					26	Tops	1.94	0.02	0.03	<0.01	<0.01	-
						35	Roots	0.11	0.01	<0.01	<0.01	<0.01	0.01
						35	Tops	2.67	0.15	0.05	<0.01	<0.01	-
Study code:	384194	Carrot	Italy	BAS 610 06 F	47	0	W. plant*	6.11	0.07	0.03	<0.01	<0.01	-
DocID:	2013/1106641			WG		21	Roots	0.46	0.05	<0.01	<0.01	<0.01	0.11
Trial No:	L110389			4 x 0.750		21	Tops	32.80	0.31	0.50	<0.01	<0.01	-
GLP:	Yes					29	Roots	0.45	0.05	<0.01	<0.01	<0.01	0.16
Year:	2011					29	Tops	20.80	0.31	0.33	<0.01	<0.01	-
						35	Roots	0.46	0.09	<0.01	<0.01	<0.01	0.23
						35	Tops	11.58	0.85	0.28	0.01	<0.01	-
Study code:	384194	Carrot	Spain	BAS 610 06 F	42	0	W. plant*	32.20	0.08	0.01	0.01	0.01	-
DocID:	2013/1106641			WG		20	Roots	0.25	0.02	<0.01	<0.01	<0.01	0.02
Trial No:	L110390			4 x 0.750		20	Tops	2.29	0.08	0.03	<0.01	<0.01	-
GLP:	Yes					27	Roots	0.15	0.01	<0.01	<0.01	<0.01	0.01
Year:	2011					27	Tops	2.12	0.08	0.02	<0.01	<0.01	-
						34	Roots	0.09	0.01	<0.01	<0.01	<0.01	0.01
						34	Tops	1.68	0.06	<0.01	<0.01	<0.01	-

0 Variation of 10% at most
1 Days after last application
2 At last application
3 RP 030228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4 RP 032490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
* Whole plants with roots

Report:	CA 6.3.1/6 Guedez-Orozco A.,Schmidt C., 2014a Determination of M610F007 (Reg.No. 5916256) and Reg. No. 85831 in plant matrices 2014/1049029
Guidelines:	none
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

This study was not yet mentioned in the application.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 610 06 F (WG),
Lot/Batch #:	08-160018, 09-160007, 09-160008, each 750 g/kg iprodione, nominal
Purity:	Not reported
CAS#:	36734-19-7 (iprodione), 626-43-7 (RP32596)
Development code:	Not reported
Spiking levels:	0.01-10.0 mg/kg

2. Test Commodity:

Crop:	Carrots, lettuce, tomato, broccoli
Type:	Root and tuber vegetables, fruiting vegetables, brassica vegetables
Variety:	Refer to summaries of studies listed in Table 6.3.1-26
Botanical name:	*Daucus carota, Lactuca sativa, Solanum lycopersicum, Brassica oleracea*
Crop parts(s) or processed commodity:	Lettuce heads, tomato fruit, broccoli inflorescences, carrot (roots, washed carrots, wash water, peeled carrots, carrot peel, cooked carrots, cooking liquid, juice, canned carrots, vegetable stock, blanching water, wet pomace)
Sample size:	Refer to summaries of studies listed in Table 6.3.1-26

B. STUDY DESIGN

1. Test procedure

The conjugate of glutamic acid and 3,5-dichloroaniline (RP32596) was identified as major metabolite M610F007 in carrot roots in the metabolism study CA 6.2.1/1. Up to this finding the compound was not known as metabolite of iprodione.
In order to find out if this metabolite is also formed in other crops rather than carrot, relevant samples of residue studies testing BAS 610 06 F between 2009 and 2011 were re-analysed for this compound. In most cases, RP32596 was also re-analysed.
In the following table the studies and matrices are shown together with the application scheme used in the respective study.

Table 6.3.1-26: Target application rates of corresponding studies

Reference	DocID	Crop	Commodity	Application scheme		
				No	Rate (kg a.s./ha)	PHI
Supervised field studies						
CA 6.3.1/4	2011/1120989	Carrot	Root	4	0.75	27
CA 6.3.1/5	2013/1106641	Carrot	Root	4	0.75	27
CA 6.3.2/5	2011/1120988	Lettuce	Head	3	0.75	21
CA 6.3.2/6	2012/1273240	Lettuce	Head	3	0.75	21
Suppl. Data*	2011/1218406	Tomato	Fruit	4	1.1	3
Suppl. Data*	2011/1120983	Tomato	Fruit	4	1.1	3
Suppl. Data*	2011/1248039	Broccoli	Inflorescence	3	0.5	14
Suppl. Data*	2011/1120987	Broccoli	Inflorescence	3	0.5	14
Processing study						
CA 6.5.3/1	2011/1248837	Carrot PF	Carrot (root, washed, peeled, blanched, cooked, canned) wash water, juice, wet pomace	4	2.25	27
Field rotational crop study						
CA 6.6.2/1	2012/1321579	Carrot	Root	1	4.05 to soil, 30 d replant interval	

*See chapter 6.3.3

2. Description of analytical procedures

The specimens were analyzed for iprodione metabolites M610F007 and RP32596 with BASF method No L0180/01, quantifying each relevant analyte with an LOQ of 0.01 mg/kg. Metabolite M610F007 is extracted with methanol. An aliquot of the extract is centrifuged, diluted with water and measured directly. RP32596 is extracted with a mixture of acetonitrile and water. An aliquot of the extract is centrifuged and measured directly. The final determination of both analytes was performed by UPLC-MS/MS.

Table 6.3.1-27: Summary of recoveries of M610F007 and RP32596

Matrix	Fortification Level [mg/kg]	RP32596				M610F007			
		Mean [%]	SD [±]	RSD [%]	n	Mean [%]	SD [±]	RSD [%]	n
Carrot root	0.01, 0.1, 1.0	85.5	2.7	3.1	5	98.7	6.1	6.2	9
Carrots, peeled	0.01, 1.0, 10	-	-	-	-	97.3	3.9	4.1	3
Carrots, juice	0.01, 0.1, 1.0	-	-	-	-	96.5	3.2	3.3	3
Blanching water	0.01, 0.1, 1.0	-	-	-	-	96.9	1.2	1.2	3
Wet pomace	0.01, 0.1, 1.0, 10	-	-	-	-	93.5	2.5	2.6	4
Vegetable stock	0.01, 0.1, 1.0	-	-	-	-	98.5	3.4	3.5	3
Lettuce head	0.01, 0.1, 1.0	80.1	14	18	5	96.0	4.2	4.3	6
Tomato	0.01, 0.1, 1.0	96.2	N/A	N/A	2	95.6	1.2	1.3	3
Broccoli inflorescence	0.01, 0.1, 1.0	105	N/A	N/A	2	93.9	2.6	2.7	3
Overall:		87.9	12	14	14	96.6	4.1	4.3	37

N/A not applicable, Calculation of SD and RSD is only applicable for three or more single values

II. RESULTS AND DISCUSSION

For the purpose of overview residue ranges for the different trials and crops are shown in Table 6.3.1-7, detailed residue levels are shown and discussed in the corresponding studies (Table 6.3.1-26).

Residues of M610F007 declined in carrot roots from 0.011-0.46 mg/kg at 0 DALA to 0.012-0.38 mg/kg at 26-29 DALA and 0.010-0.29 mg/kg after 34-35 DALA. No residues of RP32596 above the LOQ of 0.01 mg/kg were found in carrot roots.
In lettuce heads residues of M610F007 in the range of <0.01-0.010 and of RP32596 in the range of <0.01-0.012 were only found after 13-15 DALA. At later sampling events no residues above the LOQ were found in lettuce head matrices.
In tomato fruits and broccoli inflorescences no residues above the LOQ were found.

No residues at or above the LOQ (0.01 mg/kg) were found in any control sample.

Table 6.3.1-28: Summary of field trial residues

Crop	Matrix	DALA[1]	Growth stage[2]	n	Residues (mg/kg)	
					RP32596	M610F007
Carrot	Root	20-22	45-49	14	<0.010	0.011–0.46
		26-29	47-49	14	<0.010	0.012-0.38
		34-35	49	14	<0.010	0.010-0.29
Lettuce	Head	13-15	35-49	16	<0.010-0.012	<0.010-0.010
		20-21	35-50	16	<0.010	<0.010
		27-28	37-51	16	<0.010	<0.010
Tomato	Fruit	1	74-89	8	<0.010	<0.010
		3-4	74-89	8	<0.010	<0.010
		6-8	85-89	8	<0.010	<0.010
Broccoli	Inflorescence	7-8	43-48	4	<0.010	<0.010
		13-15	44-49	4	<0.010	<0.010
		20-22	49	4	<0.010	<0.010

1 Days after last application
2 At harvest

Residues of M610F007 of a rotational field crop study, where iprodione was applied pre-emergence to the soil, is presented in Table 6.3.1-29. The carrot roots showed no residues of M610F007 above the limit of quantitation 121-164 days after the application. Detailed residue levels are shown and discussed in the corresponding study (CA 6.6.2/1).

Table 6.3.1-29: Summary of residues in carrot after soil application within a rotational crop study

Crop	Matrix	DALA[1]	Growth stage	n	Residues M610F007 (mg/kg)
Carrot	Root	121-164	49	4	< 0.010

1 Day after last application

Residues of M610F007 and their transfer factors of a processing study (CA 6.5.3/1) are presented in Table 6.3.1-30. Detailed residue levels are shown and discussed in the corresponding study.

Table 6.3.1-30: Summary of residues of M610F007 in processed fractions

Matrix	Residues [mg/kg]				Transfer factor				
	L100257	L100258	L100259	L100260	L100257	L100258	L100259	L100260	Mean
Roots	0.47	0.71	0.56	1.12	-	-		-	-
Washed carrot	0.59	0.72	0.32	0.83	1.26	1.01	0.57	0.74	0.90
Wash water	< 0.010	< 0.010	< 0.010	< 0.010	-	-	-	-	-
Peeled carrots	0.18	0.48	0.61	0.37	0.38	0.68	1.09	0.33	0.62
Canned carrots	0.025	0.059	0.077	0.028	0.053	0.083	0.14	0.025	0.075
Cooked carrots	0.13	0.32	0.29	0.37	0.28	0.45	0.52	0.33	0.39
Blanching water	0.024	0.091	0.028	0.098	0.05	0.13	0.050	0.088	0.079
Cooking liquid	0.096	0.24	0.064	0.24	0.20	0.34	0.11	0.21	0.22
Carrot peel	0.74	1.6	0.97	2.9	1.57	2.25	1.73	2.59	2.0
Juice	0.32	0.67	0.27	0.81	0.68	0.94	0.48	0.72	0.71
Wet pomace	0.49	0.98	0.44	1.0	1.04	1.38	0.79	0.89	1.03
Vegetable stock	0.021	0.034	0.045	0.018	0.045	0.048	0.080	0.016	0.047

Transfer factor Residue of processed product/ residue of raw agricultural commodity

III. CONCLUSION

The study shows that metabolite M610F007 is found in amounts up to 0.46 mg/kg in carrot root at 14 DALA and degrading during the study to values between 0.01 and 0.29 mg/kg. In the other crops like lettuce, broccoli or tomato, no residues of M610F007 were found which proves that this metabolite is only found in roots.

The re-analysis of the carrot root samples of the field rotational crop study CA 6.6.2/1 proves that no residues above the limit of quantitation of metabolite M610F007 are taken up into carrots planted 30 to 33 days after application of BAS 610 06 F to bare soil.

Furthermore, the re-analysis of the processing study CA 6.5.3/1 shows that residues of M610F007 do not accumulate in processed commodities destined for human consumption. It can be seen that the residues are mainly found in peel.

CA 6.3.2 Lettuce

Table 6.3.2-1: cGAP for the use of BAS 610 F in/on lettuce

Crop	Maximum applied dose	Water volume	PHI	Application method	Application timing
Lettuce, outdoor	3 x 0.750 kg BAS 610 F/ha	200-1000 L/ha	21	spray application	BBCH 10-49
Lettuce, indoor	3 x 0.750 kg BAS 610 F/ha	200-1000 L/ha	14	spray application	BBCH 10-49

PHI = pre-harvest interval

Table 6.3.2-2: GAP information of residue trials conducted in lettuce in 2011

Region	Country (No of trials) Year	Formu-lation	Application[0]				DALA[1]	DocID	EU accepted
			Method	Rate[0] (kg a.s./ha)	Spray conc. (kg a.s./hL)	No			
Northern EU	United Kingdom (1) 1974	LFA-2043	spray appl.	0.56	0.05	3	22	C023659	yes
	Germany 1976 (1) 1977 (1)	Rovral 50% WP	spray appl.	0.750 0.500	0.125 0.083	3	0-21	R004273	yes
	United Kingdom (1) 1999	EXP01862F SC	spray appl.	0.750	0.11	3	21	R018826	no
	France (1) Germany (1) United Kingdom (1) Belgium (1) 2010	BAS 610 06 F, WG	spray appl.	0.750	0.15	3	0 14±1 21±1 28±1	2011/ 1120988	no
	France (1) Germany (1) United Kingdom (1) The Netherlands (1) 2011	BAS 610 06 F, WG	spray appl.	0.750	0.15	3	0 14±1 21±1 28±1	2012/ 1273240	no

Table 6.3.2-2: GAP information of residue trials conducted in lettuce in 2011

Region	Country (No of trials) Year	Formu-lation	Application[0]				DALA [1]	DocID	EU accepted
			Method	Rate[0] (kg a.s./ha)	Spray conc. (kg a.s./hL)	No			
EU South	Italy (1) 1996	EXP10370 A WG	spray appl.	0.750	0.08	3	21	C027043	no
	Italy (1) 1996	EXP01671 H WP	spray appl.	0.750	0.08	3	21	C027043	no
	Spain (1) France (1) Italy (1) Greece (1) 2010	BAS 610 06 F, WG	spray appl.	0.750	0.15	3	0 14±1 21±1 28±1	2011/ 1120988	no
	Spain (1) France (1) Italy (1) Greece (1) 2011	BAS 610 06 F, WG	spray appl.	0.750	0.15	3	0 14±1 21±1 28±1	2012/ 1273240	no
Indoor (EU N + S)	Germany (2) 1976	Rovral 50% WP	spray appl.	0.750	0.15	3	14	R004273	yes
	France (2) 2000	EXP01862F	spray appl.	0.740	0.15	3	14±1	C012437	no
	Germany (1) 1999	EXPO1671H	spray appl.	0.750-0.790	0.13	3	0 7 14	R018833	no
	France (2) Germany (2) The Netherlands (1) Italy (1) Greece (1) Spain (1) 2013	BAS 610 06 F, WG	spray appl.	0.750	0.15	3	0 7±1 14±1 21±1	2013/ 1311886	no

0 Actual application rates varied by 10% at most
1 Days after last application

The studies not yet evaluated are summarized in the following chapter.

Report: CA 6.3.2/1
Gateaud L., 2001b
Iprodione - Formulation EXP 01862F (SC) - Greenhouse / France / 2000 - 2 Harvest trials - Residues in lettuce (leaf)
C012437
Guidelines: EEC 91/414, EEC 96/68
GLP: yes
(certified by Groupe Interministeriel des Produits Chimiques, France)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: Iprodione BAS 61005F
Lot/Batch #: OP990926: 500 g/L Iprodione, nominal
Purity: Not reported
CAS#: 36734-19-7
Development code: Not reported
Spiking levels: 0.02, 0.20, 10.02 mg/kg

2. Test Commodity:

Crop: Lettuce
Type: Leafy vegetables
Variety: Sensai, Omega
Botanical name: *Lactuca sativa*
Crop parts(s) or processed commodity: Leaf
Sample size: 12 Lettuces, 4.4-5.5 kg (Sensai), 12 Lettuces, 5.62-6.32 kg (Omega)

B. STUDY DESIGN

1. Test procedure

During 2000 two residue trials in lettuce were carried out in greenhouse in France, to determine the residue level of Iprodione residues in or on raw agricultural commodities (RAC). EXP01862F (SC), a suspension concentrate (SC) containing 500 g/L iprodione was applied three times at a rate equivalent to 0.740 kg iprodione/ha in spray volumes of 501-629 L/ha. The foliar applications were performed 33-35 days, 23-25 days and 14-15 days before the anticipated harvest with an interval of 10 days. Specimens of lettuce were collected 14-15 days after the last application (BBCH 47-49). Samples were immediately sent to the laboratory for analysis.

Table 6.3.2-3: Target application rates and timings for lettuce

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	2	3	G	EXP01862F (SC)	BAS 610 F	0.737-0.740	501-629	14-15 days 23-25 days 33-35 days

2. Description of analytical procedures

The specimens were analyzed for iprodione (BAS 610 F) with an Aventis CropScience method No AR144-97, quantifying parent iprodione with an LOQ of 0.02 mg/kg. Residues were extracted from lettuce matrices with acetone. After addition of water and sodium chloride, a clean-up by liquid-liquid partitioning with dichloromethane and purification was conducted. The final determination of the analyte was performed by gas chromatography. The quantification was carried out by external standardization.

Table 6.3.2-4: Summary of recoveries of iprodione in/on lettuce

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
Aventis CropScience method No AR144-97		**EXP01862F (SC)**		
Lettuce leaf	0.02-10.02	7	91.71	11.57

II. RESULTS AND DISCUSSION

The residues for the different trials are shown in Table 6.3.2-5. Residues of iprodione in lettuce ranged from 8.1 to 2.5 mg/kg at in whole plant commodities after 14 and 15 DALA in leafs in two independent trials. Iprodione residues above the LOQ (0.02 mg/kg) were found in some control samples.

Table 6.3.2-5: Summary of Iprodione residues in lettuce

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Matrix	Range of residues (average) (mg/kg)
France	2010	14 -	47-49	leaf	2.5 - 8.1*

1 Days after last application
2 At harvest
* Residues in untreated samples ranged from 0.32 to 0.79 mg/kg

III. CONCLUSION

Residues of iprodione were determined in lettuce commodities during the harvesting period.

Table 6.3.2-6: Residues of iprodione in lettuce after three applications of EXP01862F in greenhouse in France

Study details		Crop	Country	Formulation application rate (kg a.s./ha))	GS[2] BBCH	DALA[1]	Matrix	Residues found (mg/kg)
Study code:	00-527	Lettuce	France (S)	EXP01862F (SC) 3 x 0.740	48-49	15	leaf	2.5
DocID:	C012437							
Trial No:	00527TL1							
GLP:	Yes							
Year:	2000							
Study code:	00-527	Lettuce	France (N)	EXP01862F (SC) 3 x 0.7370	47-49	14	leaf	8.1
DocID:	C012437							
Trial No:	00527OR1							
GLP:	Yes							
Year:	2000							

1 Days after last application
2 At harvest

Report: CA 6.3.2/2
Gateaud L., 2000c
Iprodione - Formulation EXP 01671H (WP) - Greenhouse / Germany / 1999 - 1 decline study trial - Residues in lettuce (leaf)
R018833
Guidelines: EEC 91/414, EEC 96/68
GLP: yes
(certified by Groupe Interministeriel des Produits Chimiques, France)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: EXP01671H (WP), 500 g/kg iprodione, nominal
Lot/Batch #: 0P980512 (§4 of the field part)
Purity: Not reported
CAS#: 36734-19-7
Development code: Not reported
Spiking levels: 0.02, 0.20, 4.0 mg/kg

2. Test Commodity:

Crop: Lettuce
Type: Leafy vegetables
Variety: Sensai
Botanical name: *Lactuca sativa*
Crop parts(s) or processed commodity: Leaf
Sample size: Min. 12 heads, 0.3-1.0 kg

B. STUDY DESIGN

1. Test procedure

During 2000 a residue trial in lettuce was carried out in greenhouse in Germany (North), to determine the residue level of iprodione residues in or on raw agricultural commodities (RAC). EXP01671H (ROVRAL), a wettable powder (WP) containing 500 g/kg iprodione was applied three times at a dose rate equivalent to 1.5 kg iprodione/ha in spray volumes of 600 L/ha. The foliar applications were performed at application intervals of 10 days, 22 days before the anticipated harvest (1st application, BBCH 14), 11 days before the anticipated harvest (2nd application, BBCH 41) and 0 days before the anticipated harvest (3rd application, BBCH 48). Specimens of lettuce were collected 0, 7 and 14 days after the last application (BBCH 47-49). Samples were frozen within 24 hours from sampling, then kept frozen (-18°C) during transport and storage until analysis.

Table 6.3.2-7: Target application rates and timings for lettuce

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
1999	1	3	G	EXP01671H (ROVRAL),	BAS 610 F	0.750	600	After setting of plants 10 days after 1st application 20 days after 2nd application

2. Description of analytical procedures

The specimens were analyzed for iprodione (BAS 610 F) with an Aventis CropScience method No AR144-97, quantifying parent iprodione with an LOQ of 0.02 mg/kg. Residues were extracted from lettuce matrices with acetone. After addition of water and sodium chloride, a clean-up by liquid-liquid partitioning with dichloromethane and purification was conducted. The final extracts were analyzed using gas chromatography. The quantification was carried out by external standardization.

Table 6.3.2-8: Summary of recoveries of iprodione in lettuce

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
Aventis CropScience method No AR144-97		**iprodione**		
Leafs	0.02-4.0	6	84.5	7.01

II. RESULTS AND DISCUSSION

The residue results are shown in Table 6.3.2-9. Residues of iprodione in lettuce decreased from 7.6 mg/kg to 3.4 mg/kg in whole plant commodities between day 0 and day 14. Iprodione residues above the LOQ (0.02 mg/kg) were found in control samples.

III. CONCLUSION

Residues of iprodione declined in lettuce commodities during the harvesting period. In a greenhouse trial conducted in Germany residues of iprodione declined in lettuce from 7.6 mg/kg at 0 DALA to 3.4 mg/kg at 14 DALA in leaves.

Table 6.3.2-9: Residues of iprodione in lettuce after three applications of EXPO1671H in Greenhouse in Germany

Study details		Crop	Country	Formulation application rate (kg a.s./ha))	GS BBCH	DALA[1]	Matrix	Residues found (mg/kg)
Study code:	99-634	Lettuce	Germany	EXPO1671H (WP) 1 x 0.750 1 x 0.785.5 1 x 791.0	47 48/49 49	0 7 14	leaf	7.6 4.6 3.4
DocID:	R018833							
Trial No:	99634DE1							
GLP:	Yes							
Year:	1999							

1 Days after last application
2 At harvest

Report: CA 6.3.2/3
Gateaud L., 2000d
Iprodione - Formulation EXP01862F (SC) North / United Kingdom / 1999 - 2 harvest study trials - Residues in lettuce (leaf)
R018826
Guidelines: EEC 91/414, EEC 96/68
GLP: yes
(certified by Groupe Interministeriel des Produits Chimiques, France)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: EXP01862F (SC), suspension, 500 g/L iprodione, nominal
Lot/Batch #: 0P980936
Purity: Not reported
CAS#: 36734-19-7
Development code: Not reported
Spiking levels: 0.02, 0.04, 0.2, 0.10 mg/kg

2. Test Commodity:

Crop: Lettuce
Type: Leafy vegetables
Variety: Little Gem cv Swing
Botanical name: *Lactuca sativa*
Crop parts(s) or processed commodity: Leaf
Sample size: 12 heads

B. STUDY DESIGN

1. Test procedure

During 1999 two residue trials in lettuce were carried out in United Kingdom (EU-North), to determine the residue level of Iprodione residues in or on raw agricultural commodities (RAC). EXP01862F (SC), a suspension containing 500 g/L iprodione was applied three times at a dose rate 740 to 780 g as/ha in spray volumes of 662 L/ha. The foliar applications were performed at intervals of 14 days, 49 days before the anticipated harvest (1st application, leaf development: 4-6 leaves, BBCH 14-16), 35 days before the anticipated harvest (2nd application, leaf development: 6-8 leaves, BBCH 16-18) and 21 days before the anticipated harvest (3rd application, leaf development: 10-14 leaves, BBCH 19). Mature lettuce heads were collected 21 days after the last application (BBCH 47-49). Samples were frozen within 24 hours from sampling, then kept frozen (-18°C) during transport and storage until analysis.

Table 6.3.2-10: Target application rates and timings for lettuce

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
1999	2	3	F	EXP01862F (SC)	BAS 610 F	0.750	660	Approx. 2 months before normal harvest 14 days after 1st application 28 days after 2nd application

2. Description of analytical procedures

The specimens were analyzed for iprodione (BAS 610 F) with an Aventis CropScience method No AR144-97 (modified), quantifying parent iprodione with an LOQ of 0.02 mg/kg. Residues were extracted from lettuce matrices with acetone. After addition of water and sodium chloride, a clean-up by liquid-liquid partitioning with dichloromethane and purification was conducted. The final extracts were analyzed using gas chromatography. The quantification was carried out by external standardization.

Table 6.3.2-11: Summary of recoveries of iprodione in lettuce

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
Aventis CropScience method No AR144-97		**iprodione**		
Leaf	0.02-0.2	7	88.14	7.98

II. RESULTS AND DISCUSSION

The residue ranges are shown in Table 6.3.2-14. Residues of iprodione in lettuce was found from 0.025 mg/kg to 0.11 mg/kg in plant commodities after 21 DALA in leaves of two trials. Iprodione residues were less than LOQ (0.02 mg/kg) in untreated samples, except in the 2nd plot untreated for the trial 99695GB2 (0.11 mg/kg). An inversion is suspected between the untreated samples and between the treated samples. The value obtained for 1R2 has been taken into account.

Table 6.3.2-12: Summary of iprodione residues in lettuce

Region	Year	DALA[1]	Growth stage (BBCH)	Matrix	Range of residues (average) (mg/kg)
United Kingdom	1999	21	Harvest	leaf	0.025 - 0.11*

1 Days after last application
* An inversion was suspected between the untreated samples (1R2; 0.11 mg/kg) and between the treated samples (2R1; <0.020 mg/kg) for the trial 99695GB2. The value obtained for 1R2 was taken into account..

III. CONCLUSION

Residues of iprodione in lettuce commodities during the harvesting period were at 0.025 and 0.11 mg/kg at 21 DALA.

Table 6.3.2-13: Residues of iprodione in lettuce after three applications of EXP01862F in in field trials in the United Kingdom

Study details		Crop	Country	Formulation application rate (kg a.s./ha))	GS BBCH	DALA[1]	Matrix	Residues found (mg/kg)
Study code: **DocID:** **Trial No:** **GLP:** **Year:**	99-695 R018826 99695GB1 Yes 1999	lettuce	United Kingdom	EXP01862F (SC) 1 x 0.782 1 x 0.717 1 x 0.772	harvest	21	leaf	0.025
Study code: **DocID:** **Trial No:** **GLP:** **Year:**	99-695 R018826 99695GB2 Yes 1999	lettuce	United Kingdom	EXP01862F (SC) 1 x 0.777 1 x 0.749 1 x 0.737	harvest	21	leaf	0.11*

1 Days after last application
* An inversion was suspected between the 1R2 untreated samples (0.11 mg/kg) and between the 2R1 treated samples (<0.020 mg/kg) for the trial 99695GB2. The value obtained for 1R2 was taken into account.

Report: CA 6.3.2/4
Maestracci M., 1997b
Iprodione - Formulations EXP10370A (WG) and EXP01671H (WP) - Trial Italy 1996 - Residues in lettuce
C027043
Guidelines: none
GLP: yes

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	Formulations EXP10370A (WG) and EXP01671H (WP)
Lot/Batch #:	OP971126, 500 g/kg iprodione, nominal
Purity:	Not reported
CAS#:	36734-19-7
Development code:	Not reported
Spiking levels:	0.02, 0.40 mg/kg

2. Test Commodity:

Crop:	Lettuce
Type:	Leafy vegetables
Variety:	Cappuccio
Botanical name:	*Lactuca sativa*
Crop parts(s) or processed commodity:	Leaf
Sample size:	3 kg

B. STUDY DESIGN

1. Test procedure

During the 1996 growing season two trials in lettuce were conducted in Italy, to determine the residue level of iprodione in or on raw agricultural commodities (RAC). EXP10370A (500 g/kg iprodione, WG (water dispersible granule) and EXP01671H (500 g/kg iprodione, WP (wettable powder) was applied three times at a rate equivalent to 750 g as/ha in spray volumes of 1000 L/ha (BBCH 13, 13 and 45). Specimens of lettuce were collected 21 days (BBCH 79) after the last application. Samples were stored frozen at -20°C for a maximum of 4 months until analysis.

Table 6.3.2-14: Target application rates and timings for lettuce

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
1996	1	3	F	EXP10370A (WG) and 01671H (WP)	BAS 610 F	0.750	1000	41 days 31 days 21 days before harvest

2. Description of analytical procedures

The specimens were analyzed for iprodione (BAS 610 F) using Method CNG-An 20610 (modified), quantifying iprodione with a LOQ of 0.02 mg/kg. Residues of iprodione were extracted by with acetone. After liquid-liquid partition with dichloromethane and clean-up, residues were quantified by electron-capture gas chromatography.

Table 6.3.2-15: Summary of recoveries of iprodione in lettuce

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
CNG-An 20610		BAS 610 F		
Leaf	0.02-0.40	3	109	2,4

II. RESULTS AND DISCUSSION

The residue ranges for the different formulations are shown in Table 6.3.2-16 detailed residue levels are shown in Table 6.3.2-17.
Residues of iprodione ranged from 0.17 to 0.22 mg/kg in plant commodities after 21 DALA in leaves of lettuce, respectively. Iprodione residues were less than LOQ (0.02 mg/kg) in untreated samples.

Table 6.3.2-16: Summary of iprodione residues in lettuce after application of EXP10370A (WG) and 01671H (WP)

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Matrix	Formulation	Range of residues (average)* (mg/kg)
Italy	1996	21	45	leaf	EXP10370A (WG)	0.13-0.30
					EXP01671H (WP)	0.11-0.23

1 Days after last application
2 At last application

III. CONCLUSION

Residues of iprodione in lettuce commodities during the harvesting period averaged at 0.17 and 0.22 mg/kg at 21 DALA.

Table 6.3.2-17: Residues of iprodione in lettuce after three applications of EXP10370A (WG) and 01671H (WP) in Italy

Study details		Crop	Country	Formulation application rate (kg a.s./ha)	GS [2)] BBCH	DALA[1)]	Matrix	Residues found (mg/kg)
Study code:	96-723	Lettuce	Italy, Bologna	EXP10370A (WG) 3 x 0.750	45	21	leaf	0.22
DocID:	C027043							
Trial No:	96723BO1							
GLP:	Yes			EXP01671H (WP) 3 x 0.750	45	21	leaf	0.17
Year:	1996							

1 Days after last application
2 At last treatment

Report: CA 6.3.2/5
Moreno S., 2012e
Study on the residue behaviour of BAS 610 F in lettuce, after treatment with BAS 610 06 F under field conditions in North and South Europe, season 2010
2011/1120988

Guidelines: EEC 87/18 (No. L 15/29) 1986, International guidelines for distribution and pesticides application AEPLA FAO 1985, EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5 Appendix B (July 22 1997)

GLP: yes
(certified by ENAC, Entidad Nacional de Acreditación, Madrid Spain)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 610 06 F (WG)
Lot/Batch #: 08-160018, 750 g/kg iprodione, nominal
Purity: Not reported
CAS#: 36734-19-7
Development code: Not reported
Spiking levels: 0.01, 0.1 and 10 mg/kg

2. Test Commodity:

Crop: Lettuce
Type: Leafy vegetables
Variety: Lollo Bionda, Kyrio, Lollo Bianca, Danyelle, Simson, Lorca, Gentilina Impulsion, Iris
Botanical name: *Lactuca sativa*
Crop parts(s) or processed commodity: Whole plant without roots, lettuce heads
Sample size: 0.5-8.0 kg (min. 12 plants)

B. STUDY DESIGN

1. Test procedure

During the 2010 growing season 8 trials in lettuce were conducted in different representative growing areas in the EU, 4 in the North (Germany, France, Belgium, United Kingdom) and 4 in the South (Spain, Italy, Greece, France) to determine the residue level of iprodione and its metabolites RP 30228 (Reg. No 5079647), RP 32490 (Reg. No 5079628) and RP 32596 (Reg. No 85831) in or on raw agricultural commodities (RAC).
Selected samples were also analysed for the recently discovered metabolite M610F007 in a separate study. The analytical details are described in CA 6.3.1/6, the results thereof are also included in this summary.
BAS 610 06 F (750 g/kg iprodione, WG) was applied three times at a rate equivalent to 0.750 kg iprodione/ha in a spray volume of 500 L/ha. The applications were performed at growth stages BBCH 13-16 (1st application), BBCH 16-42 (2nd application) and BBCH 33-45 (3rd application). Specimens of whole plant without roots were collected immediately after application (BBCH 33-45); lettuce heads were sampled 13-14 days (BBCH 35-49), 21 days (BBCH 35-49) and 27-28 days (BBCH 37-49) after the last application. Samples were stored frozen at or below -18°C for a maximum of 454 days until analysis.

Table 6.3.2-18: Target application rates and timings for lettuce

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
2010	8	3	F	BAS 610 06 F (WG)	BAS 610 F	0.750	500	49±1 days 35±1 days 21±1 days before harvest

2. Description of analytical procedures

The specimens were analysed for iprodione (BAS 610 F) and its metabolites RP 030228 (Reg. No 5079647), RP 032490 (Reg. No 5079628) and RP 032596 (Reg. No 85831) with BASF method No L0180/01, quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. Residues were extracted from lettuce matrices with a mixture of acetonitrile and water. An aliquot of the extract was centrifuged and measured directly. The final determination of all analytes was performed by UPLC-MS/MS.

Table 6.3.2-19: Summary of recoveries of iprodione and its metabolites in/on lettuce

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No L0180/01		**BAS 610 F**			**RP 30228 (Reg. No 5079647)**		
Whole plant*/heads	0.01, 0.10, 1.0, 10, 40	15	92.4	6.1	15	94.7	7.2
BASF method No L0180/01		**RP 32490 (Reg. No 5079628)**			**RP 32596 (Reg. No 85831)**		
Whole plant*/heads	0.01, 0.10, 10	15	95.6	5.5	15	87.3	6.6

* without roots

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown Table 6.3.2-20, detailed residue levels are shown in Table 6.3.2-21 and Table 6.3.2-22.

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Residues of iprodione in head lettuce declined during the harvesting period and were comparable in trials performed in the EU North and South.

Parent iprodione was found up to 24.8 mg/kg at the beginning of the study and declined over the time investigated to values of 0.07 and 0.06 mg/kg in the two zones. At the intended PHI of 21 days it was up to 0.48 mg/kg in North and 0.24 mg/kg in EU South.

Metabolites RP 30228 and RP 32490 occurred in comparable amounts in both zones: Starting with values up to 0.05 mg/kg in N-EU and 0.09 and 0.11 mg/kg in S-EU they degraded to residues of 0.03 and 0.06 mg/kg, respectively.

Residues of RP 32596 or M610F007 above the LOQ of 0.01 mg/kg were not detected throughout the study

No residues at or above the LOQ (0.01 mg/kg) were found in any control sample.

Table 6.3.2-20: Summary of residues in lettuce

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)					
				Matrix	iprodione	RP 30228 [3)]	RP 32490 [4)]	RP 32596	M610F007
					as parent equivalents				
Northern EU	2010	0	35-45	Wh. plant*	10.94-24.8	0.03-0.05	<0.01-0.05	<0.01	-
		13-14	46-49	Head	0.04-2.65	<0.01-0.12	<0.01-0.08	<0.01	<0.01
		21	47-50	Head	<0.01-0.48	<0.01-0.01	<0.01-0.04	<0.01	<0.01
		27-28	49-51	Head	<0.01-0.07	<0.01	<0.01-0.01	<0.01	<0.01
EU South	2010	0	33-45	Wh. plant*	2.18-24.80	<0.01-0.09	<0.01-0.11	<0.01	-
		13-14	35-47	Head	0.01-0.64	<0.01-0.06	<0.01-0.09	<0.01	<0.01
		21	35-49	Head	0.02-0.24	<0.01-0.03	<0.01-0.06	<0.01	<0.01
		27-28	37-49	Head	<0.01-0.06	<0.01-0.01	<0.01-0.02	<0.01	<0.01

1 Days after last application
2 At harvest
3 RP 030228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4 RP 032490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
* Whole plant without roots

III. CONCLUSION

Residues of iprodione declined in lettuce heads during the harvesting period and were comparable in trials performed in the EU North and South. Values started with high values of 24.8mg/kg in both zones, but were found up to 0.48 mg/kg in N-EU and up to 0.24 mg/kg in S-EU at the intended PHI of 21 days.
Metabolites RP 30228 and RP 32490 were also comparable during the study in EU North and South ranging between < 0.01 and 0.12 mg/kg. At the last sampling both were degraded to values at or slightly above LOQ.
Metabolites RP 32596 and M610F007 were below LOQ at all times.

Table 6.3.2-21: Residues of iprodione in lettuce after three applications of BAS 610 06 F (WG) in Northern Europe

Study details		Crop	Country	Formulation application Rate (kg a.s./ha)[0)]	GS[2] BBCH	DA-LA[1]	Matrix	Residues (mg/kg)				
								iprodione	RP 30228 [3)]	RP 32490 [4)]	RP 32596	M610 F007
								as parent equivalents				
Study code	384189	Lettuce	Germany	BAS 610 06 F	35	0	W. plant*	13.14	0.03	0.03	<0.01	
DocID:	2011/1120988			WG		14	Head	0.32	0.02	0.03	<0.01	<0.01
Trial No:	L100245			3 x 0.750		21	Head	0.02	<0.01	<0.01	<0.01	<0.01
GLP:	Yes					28	Head	<0.01	<0.01	<0.01	<0.01	<0.01
Year:	2010											
Study code:	384189	Lettuce	France	BAS 610 06 F	41	0	W. plant*	11.34	0.05	0.05	<0.01	
DocID:	2011/1120988			WG		13	Head	1.21	0.12	0.08	<0.01	<0.01
Trial No:	L100246			3 x 0.750		21	Head	0.04	<0.01	0.01	<0.01	<0.01
GLP:	Yes					28	Head	<0.01	<0.01	<0.01	<0.01	<0.01
Year:	2010											
Study code:	384189	Lettuce	Belgium	BAS 610 06 F	45	0	W. plant*	10.94	0.03	<0.01	<0.01	
DocID:	2011/1120988			WG		14	Head	0.04	<0.01	<0.01	<0.01	<0.01
Trial No:	L100247			3 x 0.750		21	Head	<0.01	<0.01	<0.01	<0.01	<0.01
GLP:	Yes					28	Head	<0.01	<0.01	<0.01	<0.01	<0.01
Year:	2010											
Study code:	384189	Lettuce	United	BAS 610 06 F	35-37	0	W. plant*	24.80	0.04	0.05	<0.01	
DocID:	2011/1120988		Kingdom	WG		14	Head	2.65	<0.01	0.08	<0.01	<0.01
Trial No:	L100248			3 x 0.750		21	Head	0.48	0.01	0.04	<0.01	<0.01
GLP:	Yes					27	Head	0.07	<0.01	0.01	<0.01	<0.01
Year:	2010											

0 Variation of 10% at most
1 Days after last application
2 At last application
3 RP 030228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4 RP 032490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
* Whole plants without roots

Table 6.3.2-22: Residues of iprodione in lettuce after three applications of BAS 610 06 F (WG) in Southern Europe

Study details		Crop	Country	Formulation application Rate (kg a.s./ha)[0)]	GS[2] BBCH	DA-LA[1]	Matrix	Residues (mg/kg)				
								ipro-dione	RP 30228 [3)]	RP 32490 [4)]	RP 32596	M610 F007
								as parent equivalents				
Study code	384189	Lettuce	Greece	BAS 610 06 F	33-35	0	W. plant*	24.80	0.09	0.11	<0.01	-
DocID:	2011/1120988			WG		14	Head	0.64	0.06	0.09	<0.01	<0.01
Trial No:	L100249			3 x 0.750		21	Head	0.24	0.03	0.06	<0.01	<0.01
GLP:	Yes					28	Head	0.06	0.01	0.02	<0.01	<0.01
Year:	2010											
Study code:	384189	Lettuce	Italy	BAS 610 06 F	33	0	W. plant*	12.98	0.03	0.02	<0.01	-
DocID:	2011/1120988			WG		14	Head	0.58	0.02	0.05	<0.01	<0.01
Trial No:	L100250			3 x 0.750		21	Head	0.23	0.02	0.05	<0.01	<0.01
GLP:	Yes					27	Head	0.03	<0.01	<0.01	<0.01	<0.01
Year:	2010											
Study code:	384189	Lettuce	Spain	BAS 610 06 F	45	0	W. plant*	13.56	0.02	<0.01	<0.01	-
DocID:	2011/1120988			WG		13	Head	0.64	0.01	0.03	<0.01	<0.01
Trial No:	L100251			3 x 0.750		21	Head	0.06	<0.01	<0.01	<0.01	<0.01
GLP:	Yes					28	Head	0.05	<0.01	<0.01	<0.01	<0.01
Year:	2010											
Study code:	384189	Lettuce	France	BAS 610 06 F	45	0	W. plant*	2.18	<0.01	<0.01	<0.01	-
DocID:	2011/1120988			WG		14	Head	0.01	<0.01	<0.01	<0.01	<0.01
Trial No:	L100252			3 x 0.750		21	Head	0.02	<0.01	<0.01	<0.01	<0.01
GLP:	Yes					28	Head	<0.01	<0.01	<0.01	<0.01	<0.01
Year:	2010											

0 Variation of 10% at most
1 Days after last application
2 At last application
3 RP 030228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4 RP 032490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
* Whole plants with roots

Report:	CA 6.3.2/6 Perny A., 2012b Study on the residue behaviour of BAS 610 F (Iprodione) after treatment with BAS 610 06 F in lettuce under field conditions in Northern and Southern Europe, 2011 2012/1273240
Guidelines:	EEC 1607/VI/97 rev. 2 10.06.1999, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 7029/VI/95 rev. 5 Appendix B, EEC 7525/VI/95 rev. 7
GLP:	yes (certified by Groupe Interministeriel des Produits Chimiques, France)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 610 06 F (WG)
Lot/Batch #:	09-160007, 750 g/kg iprodione, nominal
Purity:	Not reported
CAS#:	36734-19-7
Development code:	Not reported
Spiking levels:	0.01, 0.1 and 10 mg/kg

2. Test Commodity:

Crop:	Lettuce
Type:	Leafy vegetables
Variety:	Open leaf varieties: Pristalion Batavia, Caipira, Lollo Rosso, Bergamo, Manchester, Cigal, Foglia Di Quercia, Batavia
Botanical name:	*Lactuca sativa*
Crop parts(s) or processed commodity:	Whole plant without roots, lettuce heads
Sample size:	0.5-1.0 kg (12 plants)

B. STUDY DESIGN

1. Test procedure

During the 2011 growing season 8 trials in lettuce were conducted in different representative growing areas in the EU, 4 in the North (Germany, France, Netherlands, United Kingdom) and 4 in the South (Spain, Italy, Greece, France) to determine the residue level of iprodione and its metabolites RP 30228 (Reg. No 5079647), RP 32490 (Reg. No 5079628), RP 32596 (Reg. No 85831) and RP 37176 (Reg. No 5079612) in or on raw agricultural commodities (RAC).
Selected samples were also analysed for the recently discovered metabolite M610F007 in a separate study. The analytical details are described in CA 6.3.1/6, the results thereof are also included in this summary.
BAS 610 06 F (750 g/kg iprodione, WG) was applied three times at a rate equivalent to 0.750 kg iprodione/ha in a spray volume of 500 L/ha. The applications were performed at growth stages BBCH 13-18 (1st application), BBCH 16-42 (2nd application) and BBCH 33-43 (3rd application). Specimens of whole plant without roots were collected immediately after application (BBCH 33-43); lettuce heads were sampled 13-14 days (BBCH 41-49), 20-21 days (BBCH 45-49) and 27-28 days (BBCH 49) after the last application. Samples were stored frozen at or below -18°C for a maximum of 257 days until analysis.

Table 6.3.2-23: Target application rates and timings for lettuce

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
2011	8	3	F	BAS 610 06 F (WG)	BAS 610 F	0.750	500	49±1 days 35±1 days 21±1 days before harvest

2. Description of analytical procedures

The specimens were analysed for iprodione (BAS 610 F) and its metabolites RP 30228 (Reg. No 5079647), RP 32490 (Reg. No 5079628), RP 32596 (Reg. No 85831) and RP 37176 (Reg. No 5079612) with BASF Method No L0180/01, quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. Residues were extracted from lettuce matrices with a mixture of acetonitrile and water. An aliquot of the extract was centrifuged and measured directly. The final determination of all analytes was performed by UPLC-MS/MS.

Table 6.3.2-24: Summary of recoveries of iprodione and its metabolites in/on lettuce

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No L0180/01		**BAS 610 F**			**RP 30228 (Reg. No 5079647)**		
Whole plant*	0.01, 0.10, 10	3	100	14	3	97.2	6.2
Head**	0.01, 0.10, 10	12	103	7.2	12	100	8.4
BASF method No L0180/01		**RP 32490 (Reg. No 5079628)**			**RP 32596 (Reg. No 85831)**		
Whole plant*	0.01, 0.10, 10	3	91.1	13	3	93.5	4.5
Head**	0.01, 0.10, 10	12	97.6	9.9	12	93.4	6.2
BASF method No L0180/01		**RP 37176 (Reg. No 5079612)**					
Whole plant*	0.01, 0.10, 10	3	89.7	6.5			
Head**	0.01, 0.10, 10	12	105	10			

* Whole plant without roots
** Open leaf varieties

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.2-25, detailed residue levels are shown in Table 6.3.2-26 and Table 6.3.2-27.
Residues of parent BAS 610 F ranged from 14-31 mg/kg and degraded to a maximum of 1.4 mg/kg in the North and to 0.08 mg/kg in the South. At the intended PHI of 21 days, iprodione range between 0.05 and 1.6 mg/kg in the North and between LOQ and 0.3 mg/kg in the South.
Residues of metabolite RP 30228 were found between 0.021 and 0.12 mg/kg at the beginning and decreased to < 0.01 mg/kg in all but one trial (UK 0.06 at 28 DALA).
Residues of metabolite RP 32490 ranged from 0.01 to 0.083 mg/kg at day 0. In most trial these residues decreased during the course of the study. However, in the trials conducted in the UK and Spain a slight increase was observed.
In one trial (DE), metabolite RP 32596 was detected at the LOQ on day 0. Apart from this RP 32596 occurred in the UK trial between 0.01 and 0.02 mg/kg
Residues of RP 37176 were <0.01 mg/kg in all samples.
No residues at or above the LOQ (0.01 mg/kg) were found in any control sample.

Table 6.3.2-25: Summary of residues in lettuce

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)						
				Matrix	iprodione	RP 30228 3)	RP 32490 4)	RP 32596	RP 37176	M610 F007
					as parent equivalents					
N-EU	2011	0	33-45	Wh.plant*	14 - 22	0.028-0.12	0.025-0.055	<0.01-0.01	<0.01	-
		13-14	41-49	Head**	0.24-3.2	0.023-0.13	0.027-0.058	<0.01-0.02	<0.01	<0.01-0.01
		20-21	45-49	Head**	0.052-1.6	<0.01-0.079	0.01-0.074	<0.01-0.01	<0.01	<0.01
		27-28	49	Head**	<0.01-1.4	<0.01-0.063	<0.01-0.064	<0.01-0.01	<0.01	<0.01
S-EU	2011	0	33-45	Wh.plant*	17 - 31	0.021-0.083	0.01-0.083	<0.01	<0.01	-
		13-14	41-49	Head**	0.072-1.0	0.017-0.047	0.020-0.039	<0.01	<0.01	<0.01
		20-21	45-49	Head**	<0.01-0.3	<0.01	<0.01-0.022	<0.01	<0.01	<0.01
		27-28	49	Head**	<0.01-0.08	<0.01	<0.01-0.051	<0.01	<0.01	<0.01

1 Days after last application
2 At harvest
3 RP 30228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
5 RP 32490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
* Whole plant without roots
** Open leaf varieties

III. CONCLUSION

Residues of iprodione and its metabolites in open variety lettuce declined during the harvesting period and were comparable in trials performed in the EU North and South.
Residues of RP 32596 above LOQ were only found in the trial performed in the UK. Metabolite RP 37176 was not detected.

Table 6.3.2-26: Residues of iprodione in lettuce after three applications of BAS 610 06 F (WG) in Northern Europe

Study details		Crop	Country	Formulation application Rate (kg a.s./ha)[0)]	GS[2] BBCH	DA-LA[1]	Matrix	Residues (mg/kg)					
								iprodione	RP 30228[3)]	RP 32490[4)]	RP 32596	RP 37176	M610 F007
								as parent equivalents					
Study code	384190	Lettuce	Germany	BAS	35	0	W.plant*	14	0.12	0.055	0.01	<0.01	-
DocID:	2012/1273240			610 06 F		14	Head**	0.24	0.023	0.027	<0.01	<0.01	<0.01
Trial No:	L110447			WG		21	Head**	0.052	<0.01	0.012	<0.01	<0.01	<0.01
GLP:	Yes			3 x 0.750		27	Head**	0.015	<0.01	<0.01	<0.01	<0.01	<0.01
Year:	2011												
Study code:	384190	Lettuce	France	BAS	43	0	W.plant*	17	0.037	0.025	<0.01	<0.01	-
DocID:	2012/1273240			610 06 F		13	Head**	0.49	0.023	0.035	<0.01	<0.01	<0.01
Trial No:	L110448			WG		20	Head**	0.079	<0.01	0.01	<0.01	<0.01	<0.01
GLP:	Yes			3 x 0.750		27	Head**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Year:	2011												
Study code:	384190	Lettuce	The	BAS	45	0	W.plant*	14	0.028	0.033	<0.01	<0.01	-
DocID:	2012/1273240		Nether-	610 06 F		13	Head**	0.61	0.037	0.048	<0.01	<0.01	<0.01
Trial No:	L110449		lands	WG		21	Head**	0.058	0.01	0.013	<0.01	<0.01	<0.01
GLP:	Yes			3 x 0.750		28	Head**	0.019	<0.01	<0.01	<0.01	<0.01	<0.01
Year:	2011												
Study code:	384190	Lettuce	United	BAS	33	0	W.plant*	22	0.061	0.052	<0.01	<0.01	-
DocID:	2012/1273240		Kingdom	610 06 F		13	Head**	3.2	0.13	0.058	0.020	<0.01	0.01
Trial No:	L110450			WG		21	Head**	1.6	0.079	0.074	0.014	<0.01	<0.01
GLP:	Yes			3 x 0.750		28	Head**	1.4	0.063	0.064	0.011	<0.01	<0.01
Year:	2011												

0 Variation of 10% at most
1 Days after last application
2 At last application
3 RP 030228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4 RP 032490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
* Whole plants without roots
** Open leaf varieties

Table 6.3.2-27: Residues of iprodione in lettuce after three applications of BAS 610 06 F (WG) in Southern Europe

Study details		Crop	Country	Formulation application Rate (kg a.s./ha)[0)]	GS[2] BBCH	DA-LA[1]	Matrix	Residues (mg/kg)					
								iprodione	RP 30228[3)]	RP 32490[4)]	RP 32596	RP 37176	M610 F007
								as parent equivalents					
Study code	384190	Lettuce	Greece	BAS 610 06 F	33	0	W. plant*	31	0.083	0.083	<0.01	<0.01	
DocID:	2012/1273240			WG		14	Head**	1.0	0.024	0.039	<0.01	<0.01	<0.01
Trial No:	L110451			3 x 0.750		21	Head**	0.30	<0.01	0.022	<0.01	<0.01	<0.01
GLP:	Yes					28	Head**	0.08	<0.01	0.012	<0.01	<0.01	<0.01
Year:	2011												
Study code:	384190	Lettuce	Spain	BAS 610 06 F	41-42	0	W. plant*	19	0.078	0.044	<0.01	<0.01	
DocID:	2012/1273240			WG		14	Head**	0.072	0.017	0.020	<0.01	<0.01	<0.01
Trial No:	L110452			3 x 0.750		20	Head**	0.037	<0.01	0.012	<0.01	<0.01	<0.01
GLP:	Yes					28	Head**	<0.01	<0.01	0.051	<0.01	<0.01	<0.01
Year:	2011												
Study code:	384190	Lettuce	Italy	BAS 610 06 F	39	0	W. plant*	17	0.021	0.01	<0.01	<0.01	
DocID:	2012/1273240			WG		15	Head**	0.18	0.047	0.03	<0.01	<0.01	<0.01
Trial No:	L110453			3 x 0.750		21	Head**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
GLP:	Yes					27	Head**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Year:	2011												
Study code:	384190	Lettuce	France	BAS 610 06 F	43	0	W. plant*	20	0.055	0.029	<0.01	<0.01	
DocID:	2012/1273240			WG		14	Head**	0.17	0.022	0.030	<0.01	<0.01	<0.01
Trial No:	L110454			3 x 0.750		21	Head**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
GLP:	Yes					28	Head**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Year:	2011												

0 Variation of 10% at most
1 Days after last application
2 At last application
3 RP 030228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4 RP 032490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
* Whole plants without roots
** Open leaf varieties

Report:	CA 6.3.2/7 Plier S., 2014a Determination of residues of BAS 610 F (Iprodione) in lettuce (greenhouse) after three applications of BAS 610 06 F in Germany, France (North), France (South), Greece, Italy, Spain and The Netherlands, 2013 2014/1094138
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414, EEC 7525/VI/95 rev. 9 (March 2011), OECD 509 Crop Field Trial (2009), EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 7029/VI/95 rev. 5 Appendix B (July 22 1997)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landesentwicklung, Dresden, Germany)

The study was listed in the application under the DocID 2013/1311886. In the course of the report finalisation a new DocID was assigned.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 610 06 F (WG)
Lot/Batch #:	16-000034, 750 g/kg iprodione, nominal
Purity:	74.2 % (analyzed)
CAS#:	36734-19-7
Development code:	Not reported
Spiking levels:	0.01, 0.1, 10 and 100 mg/kg

2. Test Commodity:

Crop:	Lettuce
Type:	Leafy vegetables
Variety:	Open leaf varieties: Lolo Bionda (Bartimer), Lolo Livorno, Kinshasa RZ, Manchester, Concorde RZ, Paradai RZ, Gala, Trophy
Botanical name:	*Lactuca sativa, Valerianella locusta*
Crop parts(s) or processed commodity:	Whole plant without roots, lettuce heads
Sample size:	0.5-4.0 kg

B. STUDY DESIGN

1. Test procedure

During the 2013 growing season 8 trials on lettuce (greenhouse) were conducted in different representative growing areas in the EU, 4 in the North (Germany, France, The Netherlands) and 4 in the South (Spain, Italy, Greece, France) to determine the residue level of iprodione and its metabolites RP 30228 (Reg. No 5079647), RP 32490 (Reg. No 5079628), RP 32596 (Reg. No 85831) RP 37176 (Reg. No 5079612) and M610F007 (Reg. No. 5916256) in or on raw agricultural commodities (RAC).
BAS 610 06 F (WG) was foliar applied three times at a target application rate of 0.75 kg a.s./ha in a spray volume of 500 L/ha. The applications were performed at growth stages BBCH 11-33 (1st application), BBCH 12-45 (2nd application) and BBCH 19-47 (3rd application). Specimens of whole plant without roots and lettuce (lamb or head, respectively) were collected immediately after application (BBCH 19/31-47); after 6-8 days (BBCH 35/37-48), 13-14 days (BBCH 48-49) and 19-21 days (BBCH 49) after the last application. Samples were stored frozen at or below -18°C for a maximum of 258 days until analysis. The storage stability for RP 32596 and M610F007 is being investigated in an ongoing storage stability study.

Table 6.3.2-28: Target application rates and timings for lettuce

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	3	G	BAS 610 06 F (WG)	BAS 610 F	0.750	500	42±1 days 28±1 days 14±1 days before harvest

2. Description of analytical procedures

The specimens were analyzed for residues of BAS 610 F (Iprodione) and its metabolites RP 30228, RP 32490, RP 32596, RP 37176 and M610F007 using BASF method No L0180/01, quantifying each analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. BAS 610 F and its metabolites RP 32490, RP 30228, RP 32596 and RP 37176 were extracted with a mixture of acetonitrile and water. An aliquot of the extract is centrifuged and measured directly. M610F007 is extracted with methanol. An aliquot of the extract is centrifuged, diluted with water and measured directly. The final determination of all analytes was performed by LC-MS/MS.

Table 6.3.2-29: Summary of recoveries of iprodione and its metabolites in/on lettuce

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No L0180/01		**BAS 610 F**					
Head	0.01, 0.10, 10, 100	10	98.8	7.8			
BASF method No L0180/01		**RP 30228 (Reg. No 5079647)**			**RP 32596 (Reg. No 85831)**		
Head	0.01, 0.10, 10	9	98.3	7.7	9	96.2	6.2
BASF method No L0180/01		**RP 32490 (Reg. No 5079628)**			**RP 37176 (Reg. No 5079612)**		
Head	0.01, 0.10, 10	9	72.4	2.6	9	89.9	2.8
BASF method No L0180/01		**M610F007 (Reg. No. 5916256)**					
Head	0.01, 0.10, 10	9	97.8	6.1			

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.2-30, detailed residue levels are shown in Table 6.3.2-31.

Head lettuce: Residues of parent BAS 610 F in head lettuce ranged from 19 to 36 mg/kg at 0 DALA and decreased to a range of 0.53 and 15 mg/kg at the intended harvest of 14 DALA. At harvest of 14 DALA, residues of the metabolites PR 30228 and RP 32490 ranged from 0.069 to 0.28 mg/kg and from 0.034 to 0.083 mg/kg, respectively and were below the limit of quantitation (<0.01 mg/kg) for the metabolites RP 32596, RP 37176 and M610F007.

Lamb´s lettuce: In the two residue trials, residues of parent BAS 610 F in lamb´s lettuce ranged from 21 to 53 mg/kg at 0 DALA and decreased to a range of 4.6 and 11 mg/kg at harvest (14 DALA). At harvest, residues for the metabolite RP 30228 were between 0.58 and 1.0 mg/kg, for RP 32490 between 0.034 to 0.050 mg/kg, for RP 32596 between <0.01 and 0.019, for RP 37176 between <0.01 and 0.014 mg/kg and between 0.016 and 0.048 mg/kg for the metabolite M610F007.

In the control samples no residues at or above the limit of quantitation were found. There were five exceptions and residues of BAS 610 F (iprodione) were found. The residues in the control samples were caused by unknown contamination.

Table 6.3.2-30: Summary of residues in lettuce (greenhouse)

Matrix	DALA[1]	Growth stage[2] (BBCH)	n	Range of residues (mg/kg)					
				Iprodione	RP 30228[3]	RP 32490[4]	RP 32596	RP 37176	M610 F007
				as parent equivalents					
Head**	0	19/31-47	6	0.18 - 36	<0.010 - 0.33	<0.010 - 0.051	<0.010	<0.010 - 0.012	<0.010
Head**	6-8	35/37-48	6	1.5 - 24	0.10 - 0.57	0.042 - 0.11	<0.010 - 0.010	<0.010	<0.010
Head**	13-14	47/49-49	6	0.53 - 15	0.069 - 0.28	0.034 - 0.083	<0.010	<0.010	<0.010
Head**	19-21	49	6	0.043 - 10	0.020 - 0.13	0.017 - 0.055	<0.010	<0.010	<0.010
Lamb/ Wh.plant*	0	41/43-46	2	21 - 53	0.52 - 2.0	0.031 - 0.039	<0.010	0.016 - 0.028	0.013 - 0.015
Lamb/ Wh.plant*	7	43/45-47	2	8.8 - 20	0.96 - 2.0	0.038 - 0.053	<0.010 - 0.010	0.016 - 0.027	0.028 - 0.032
Lamb/ Wh.plant*	13	49	2	4.6 - 11	0.58 - 1.0	0.034 - 0.050	<0.010 - 0.019	<0.010 - 0.014	0.016 - 0.048
Lamb/ Wh.plant*	21	49	2	0.88 - 9.3	0.26 - 0.52	<0.010 - 0.054	<0.010 - 0.026	<0.010	<0.010 - 0.080

1 Days after last application
2 At harvest
3 RP 30228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4 RP 32490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
* Whole plant without roots
** Open leaf varieties

III. CONCLUSION

Comparing open head lettuce varieties and lamb´s lettuce in this greenhouse residue study led to the following results: With residue ranges between 0.53 - 15 mg/kg in head lettuce and 4.6 and 11 mg/kg in lamb´s lettuce parent iprodione was found over a wide range but in comparable levels in both lettuce types.

The amounts of RP 30228 in head lettuce were with levels between 0.069 - 0.28 mg/kg lower than with 0.58 and 1.0 mg/kg in lamb´s lettuce. Metabolite RP 32490 was found in similar amounts up to 0.083 mg/kg or 0.053 mg/kg, respectively.

The metabolites RP 32596, RP 37176 and M610F007 which were below the limit of quantitation in head lettuces were found in lamb´s lettuce samples: RP 32596 up to 0.026 mg/kg, RP 037176 up to 0.014 mg/kg and M610F007 up to 0.08 mg/kg.

In general, it can be concluded that the ratio of metabolites is higher in lamb´s lettuce than in head lettuce.

Table 6.3.2-31: Residues of iprodione in lettuce after three applications of BAS 610 06 F (WG) in Northern and Southern Europe (greenhouse)

Study details		Crop	Country	Formulation application Rate (kg a.s./ha)[0]	GS[2] BBCH	DA-LA[1]	Matrix	Residues (mg/kg)					
								Iprodione	RP 30228[3]	RP 32490[4]	RP 32596	RP 37176	M610 F007
								as parent equivalents					
Study code	384191	Lettuce	Germany	BAS	47	0	Head**	24	0.14	0.051	<0.01	<0.01	<0.01
DocID:	2014/1094138			610 06 F		7	Head**	19	0.10	0.049	<0.01	<0.01	<0.01
Trial No:	L130585			WG		13	Head**	15	0.081	0.052	<0.01	<0.01	<0.01
GLP:	Yes			3 x 0.750		21	Head**	3.9	0.020	0.018	<0.01	<0.01	<0.01
Year:	2013												
Study code:	384191	Lettuce	France	BAS	43	0	Head**	19	0.10	0.018	<0.01	<0.01	<0.01
DocID:	2014/1094138			610 06 F		7	Head**	8.9	0.57	0.072	0.01	<0.01	<0.01
Trial No:	L130586			WG		14	Head**	4.1	0.28	0.061	<0.01	<0.01	<0.01
GLP:	Yes			3 x 0.750		21	Head**	0.58	0.093	0.028	<0.01	<0.01	<0.01
Year:	2013												
Study code:	384191	Lettuce	Germany	BAS	46	0	W. Plant*	53	0.52	0.031	<0.01	0.016	0.015
DocID:	2014/1094138	(lamb)		610 06 F		7	W. Plant*	20	0.96	0.053	<0.01	0.016	0.032
Trial No:	L130591			WG		13	W. Plant*	11	0.58	0.050	0.019	<0.01	0.048
GLP:	Yes			3 x 0.750		21	W. Plant*	9.3	0.52	0.054	0.026	<0.01	0.080
Year:	2013												
Study code:	384191	Lettuce	The	BAS	41	0	W. Plant*	21	2.0	0.039	<0.01	0.028	0.013
DocID:	2014/1094138	(lamb)	Nether-	610 06 F		7	W. Plant*	8.8	2.0	0.038	0.01	0.027	0.028
Trial No:	L130592		lands	WG		13	W. Plant*	4.6	1.0	0.034	<0.01	0.014	0.016
GLP:	Yes			3 x 0.750		21	W. Plant*	0.88	0.26	<0.01	<0.01	<0.01	<0.01
Year:	2013												
Study code	384191	Lettuce	Greece	BAS	19/31	0	Head**	24	0.14	0.049	<0.01	0.011	<0.01
DocID:	2014/1094138			610 06 F		8	Head**	1.5	0.13	0.107	<0.01	<0.010	<0.01
Trial No:	L130588			WG		14	Head**	0.53	0.069	0.071	<0.01	<0.010	<0.01
GLP:	Yes			3 x 0.750		21	Head**	0.043	0.049	0.017	<0.01	<0.010	<0.01
Year:	2013												
Study code:	384191	Lettuce	Spain	BAS	45	0	Head**	36	0.18	0.041	<0.01	0.012	<0.01
DocID:	2014/1094138			610 06 F		6	Head**	22	0.11	0.042	<0.01	<0.01	<0.01
Trial No:	L130590			WG		14	Head**	15	0.070	0.054	<0.01	<0.01	<0.01
GLP:	Yes			3 x 0.750		20	Head**	10	0.062	0.055	<0.01	<0.01	<0.01
Year:	2013												
Study code:	384191	Lettuce	Italy	BAS	43	0	Head**	0.19	<0.01	<0.01	<0.01	<0.01	<0.01
DocID:	2014/1094138			610 06 F		6	Head**	24	0.11	0.063	<0.01	<0.01	<0.01
Trial No:	L130589			WG		13	Head**	14	0.14	0.083	<0.01	<0.01	<0.01
GLP:	Yes			3 x 0.750		19	Head**	5.3	0.099	0.055	<0.01	<0.01	<0.01
Year:	2013												
Study code:	384191	Lettuce	France	BAS	47	0	Head**	17	0.33	0.046	<0.01	<0.01	<0.01
DocID:	2014/1094138			610 06 F		7	Head**	11	0.16	0.042	<0.01	<0.01	<0.01
Trial No:	L130587			WG		14	Head**	7.8	0.16	0.034	<0.01	<0.01	<0.01
GLP:	Yes			3 x 0.750		20	Head**	7.7	0.13	0.033	<0.01	<0.01	<0.01
Year:	2013												

0 Variation of 10% at most
1 Days after last application
2 At last application
3 RP 30228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4 RP 32490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
* Whole plant without roots
** Open leaf varieties

CA 6.3.3 Supplementary Information

In addition to the residue data for the representative crops carrot and lettuce the results of other supervised field trials are shown in this chapter. These data are not meant to be fully evaluated but they provide further data on determination of the metabolites RP 30228, RP 32490 and RP 32596. These data were also considered for determination of metabolite relevance.

CA 6.3.3.1 Supervised field trials in further crops

Broccoli

Table 6.3.3.1-1: cGAP for the use of BAS 610 F in/on broccoli

Crop	Maximum applied dose	Water volume	PHI	Application method	Application timing
Broccoli	2 x 0.750 kg BAS 610 F/ha	500-800 L/ha	21	spray application	BBCH 11-49

PHI = pre-harvest interval

Table 6.3.3.1-2: GAP information of residue trials conducted in broccoli in 2009-2010 in Northern Europe

Region	Country (No of trials) Year	Formulation	Application[0]				DALA[1]
			Method	Rate[0] (kg a.s./ha)	Spray conc. (kg a.s./hL)	No	
Northern EU	Germany (1) France (1) 2009	BAS 610 06 F (WG)	spray application	0.500	0.12	2	0 7 ±1 14-±1 21-±1
	Germany (1) Netherlands (1) 2010	BAS 610 06 F (WG)	spray application	0.500	0.12	2	0 7 ±1 14-±1 21-±1

Moreno S. (2010a)

Full study reference: Moreno S. (2010a): Study on the residue behaviour of Iprodione in broccoli after treatment with BAS 610 06 F under field conditions in North Europe, season 2009,
BASF DocID 2010/1007192

Richter M. (2011a)

Full study reference: Richter M. (2011a): Determination of residues of RP 32490 (Reg.No. 5079628), RP 30228 (Reg.No. 5079647) and RP 32596 (Reg.No. 85831) in broccoli after treatment with BAS 610 06 F under field conditions in North Europe, season 2009,
BASF DocID 2011/1248039

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**

Description:	BAS 610 06 F (WG)
Lot/Batch #:	08-160018, 750 g/kg iprodione, nominal
Purity:	Not reported
CAS#:	36734-19-7
Development code:	Not reported
Spiking levels:	0.01, 0.10, 10.0

2. **Test Commodity:**

Crop:	Broccoli
Type:	Brassica vegetables
Variety:	Marathon, Monaco
Botanical name:	*Brassica oleracea*
Crop parts(s) or processed commodity:	Inflorescences
Sample size:	0.5-2.5 kg (12 plants)

B. STUDY DESIGN

1. Test procedure

During the 2009 growing season 2 trials in broccoli were conducted in different representative growing areas in the EU (North), to determine the residue level of iprodione and its metabolites RP 30228 (Reg. No 5079647), RP 32490 (Reg. No 5079628) and RP 32596 (Reg. No 85831) in or on raw agricultural commodities (RAC). BAS 610 06 F (750 g/kg iprodione, WG) was applied three times at a rate equivalent to 0.500 kg iprodione/ha in spray volumes of 400 L/ha. The applications were performed 42±1 days before the anticipated harvest (1st application, BBCH 16-21), 28±1 days before the anticipated harvest (2nd application, BBCH 31-41) and 14±1 days before the anticipated harvest (3rd application, BBCH 41-45). Specimens of inflorescences were collected immediately after the last application (BBCH 41-45). Additional samples were taken 7 days (BBCH 43-48), 13-14 days (BBCH 47-49) and 20-21 days (BBCH 49) after the last application. Samples were stored frozen at or below -18°C for a maximum of 26 months until analysis.

Table 6.3.3.1-3: Target application rates and timings for broccoli

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
2009	2	3	F	BAS 610 06 F (WG)	BAS 610 F	0.500	400	42 (±1) days 28 (±1) days 14 (±1) days before harvest

2. Description of analytical procedures

The specimens were analysed for iprodione (BAS 610 F) with BASF Method No 543/0 (L0022/01), quantifying the analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. Residues of iprodione were extracted with a mixture of acetone, water and hypochloric acid. An aliquot of the extract is centrifuged and partioned against cyclohexane. The final determination of all analytes was performed by HPLC-MS/MS.
The metabolites RP 30228 (Reg. No 5079647), RP 32490 (Reg. No 5079628) and RP 32596 (Reg. No 85831) were analysed with BASF Method No L0180/01, quantifying each relevant analyte with a LOQ of 0.01 mg/kg. Residues were extracted from broccoli matrices with a mixture of acetonitrile and water. An aliquot of the extract was centrifuged and measured directly. The final determination of all analytes was performed by UPLC-MS/MS.

Table 6.3.3-4: Summary of recoveries of iprodione and its metabolites in/on broccoli

Matrix	Fortification Level (mg/kg)	Summary Recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF Method No 543/01		**BAS 610 F**					
Inflorescences	0.01, 0.10, 10	3	96.2	12			
BASF Method No L0180/01		**RP 032490 (Reg. No 5079628)**			**RP 030228 (Reg. No 5079647)**		
Inflorescences	0.01, 0.10, 10	6	85.1	2.7	6	80	11
BASF Method No L0180/01		**RP 032596 (Reg. No 85831)**					
Inflorescences	0.01, 0.10, 10	6	77.1	5.3			

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.3.1-6, detailed residue levels are shown in Table 6.3.3.1-6.
Residues of iprodione declined in broccoli from 1.50-2.30 mg/kg at 0 DALA to 0.03-0.08 mg/kg after 13-14 DALA. There were no residues of the metabolites RP 30228, RP 32490 and RP 32596 (Reg. No 85831) found in broccoli, except RP 32490 after 7 DALA in France were 0.01 mg/kg was quantified.
No residues at or above the LOQ (0.01 mg/kg) were found in any control sample.

Table 6.3.3.1-5: Summary of residues in broccoli

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)					
				Matrix	iprodione	RP 30228 [3)]	RP 32490 [4)]	RP 32596	M610 F007
					as parent equivalents				
Northern EU	2009	0	41-45	inflorescences	1.50-2.30	<0.01	<0.01	<0.01	-
		7	43-48	inflorescences	0.11-0.23	<0.01-0.01	<0.01	<0.01	<0.01
		13-14	47-49	inflorescences	0.03-0.08	<0.01	<0.01	<0.01	<0.01
		20-21	49	inflorescences	<0.01-0.02	<0.01	<0.01	<0.01	<0.01

1 Days after last application
2 At harvest
3 RP 30228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4 RP 32490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146

III. CONCLUSION

Residues of iprodione declined in broccoli inflorescences during the harvesting period. In the EU North residues in broccoli inflorescences at the commercial harvest times ranged from 0.03-0.08 mg/kg for parent BAS 610 F. Metabolite RP 30228 was found at the LOQ of 0.01 mg/kg. Residues of the other metabolites RP 32490, RP 32596 and M610F007 above the LOQ were not detected.

Table 6.3.3.1-6: Residues of iprodione in broccoli after three applications of BAS 610 06 F (WG) in Northern Europe

Study details		Crop	Country	Formulation application Rate (kg a.s./ha)[0)]	GS[2] BBCH	DA-LA[1]	Matrix	Residues (mg/kg)				
								iprodione	RP 30228 [3)]	RP 32490 [4)]	RP 32596	M610 F007
								as parent equivalents				
Study code	357852	broccoli	DE	BAS 610 06 F WG 3 x 0.500	41-45	0	infl.	2.30	<0.01	<0.01	<0.01	-
DocID:	2010/1007192					7	infl.	0.23	0.01	<0.01	<0.01	<0.01
	2011/1248039					13	infl.	0.08	<0.01	<0.01	<0.01	<0.01
Trial No:	L090297					20	infl.	0.02	<0.01	<0.01	<0.01	<0.01
GLP:	yes											
Year	2009											
Study code:	357852	broccoli	FR	BAS 610 06 F WG 3 x 0.500	41-45	0	infl.	1.50	<0.01	<0.01	<0.01	-
Doc ID:	2010/1007192					7	infl.	0.11	<0.01	<0.01	<0.01	<0.01
	2011/1248039					14	infl.	0.03	<0.01	<0.01	<0.01	<0.01
Trial No:	L090298					21	infl.	<0.01	<0.01	<0.01	<0.01	<0.01
GLP:	yes											
Year:	2009											

1) days after last application
2) at harvest
3) RP 30228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 32490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
infl. inflorescences

Moreno S. (2012a)

Full study reference: Moreno S. (2012a): Study on the residue behaviour of Iprodione in broccoli after treatment with BAS 610 06 F under field conditions in North Europe, season 2010
BASF DocID 2011/1120987

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 610 06 F (WG)
Lot/Batch #:	08-160018, 750 g/kg iprodione, nominal
Purity:	Not reported
CAS#:	36734-19-7
Development code:	Not reported
Spiking levels:	0.01, 0.10, 10.0

2. Test Commodity:

Crop:	Broccoli
Type:	Brassica vegetables
Variety:	Ironman
Botanical name:	*Brassica oleracea*
Crop parts(s) or processed commodity:	Whole plant without roots, **i**nflorescences
Sample size:	0.5-2.5 kg (12 plants)

B. STUDY DESIGN

1. Test procedure

During the 2010 growing season 2 trials in broccoli were conducted in different representative growing areas in the EU (North), to determine the residue level of iprodione and its metabolites RP30228 (Reg. No 5079647), RP32490 (Reg. No 5079628) and RP32596 (Reg. No 85831) in or on raw agricultural commodities (RAC). Selected samples were also analysed for the recently discovered metabolite M610F007 in a separate study. The analytical details are described in CA 6.3.1/6, the results thereof are also included in this summary.
BAS 610 06 F (750 g/kg iprodione, WG) was applied three times at a rate equivalent to 0.500 kg iprodione/ha in spray volumes of 400 L/ha. The applications were performed 42±1 days before the anticipated harvest (1st application, BBCH 16-19), 28±1 days before the anticipated harvest (2nd application, BBCH 26-41) and 14±1 days before the anticipated harvest (3rd application, BBCH 42-43). Specimens of whole plant without roots were collected immediately after the last application (BBCH 42-43). Additional samples were taken 8 days (BBCH 43-45), 15 days (BBCH 44-47) and 20-22 days (BBCH 49) after the last application. Samples were stored frozen at or below -18°C for a maximum of 415 days until analysis.

Table 6.3.3.1-7: Target application rates and timings for broccoli

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
2010	2	3	F	BAS 610 06 F (WG)	BAS 610 F	0.500	400	42 (±1) days 28 (±1) days 14 (±1) days before harvest

2. Description of analytical procedures

The specimens were analysed for iprodione (BAS 610 F) and its metabolites RP 30228 (Reg. No 5079647), RP 32490 (Reg. No 5079628) and RP 32596 (Reg. No 85831) with BASF Method No L0180/01, quantifying each relevant analyte with a LOQ of 0.01 mg/kg. Residues were extracted from broccoli matrices with a mixture of acetonitrile and water. An aliquot of the extract was centrifuged and measured directly. The final determination of all analytes was performed by UPLC-MS/MS.

Table 6.3.3.1-8: Summary of recoveries of iprodione and its metabolites in/on broccoli

Matrix	Fortification Level (mg/kg)	Summary Recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF Method No L0180/01		**BAS 610 F**			**RP 32490 (Reg. No 5079628)**		
Inflorescences /whole plant	0.01, 0.10, 10	4	87.7.2	8.3	4	83.4	16.9
BASF Method No L0180/01		**RP 30228 (Reg. No 5079647)**			**RP 32596 (Reg. No 85831)**		
Inflorescences /whole plant	0.01, 0.10, 10	4	89.3	9.2	4	89.7	7.2

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.3.1-9, detailed residue levels are shown in Table 6.3.3.1-10.
Residues of iprodione declined in broccoli from 5.71-8.89 mg/kg at 0 DALA to <0.01-0.01 mg/kg after 15 DALA. There were no residues of the metabolites RP 30228, RP 32490 and RP 32596 (Reg. No 85831) found in broccoli, except RP 32490 and RP 30228 after 0 DALA were 0.02 mg/kg was quantified.
No residues at or above the LOQ (0.01 mg/kg) were found in any control sample.

Table 6.3.3.1-9: Summary of residues in broccoli

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Matrix	Range of residues (mg/kg)				
					iprodione	RP 30228 3)	RP 32490 4)	RP 32596	M610 F007
					as parent equivalents				
Northern EU	2010	0	41-43	whole plant*	5.71-5.89	<0.01-0.02	0.02	<0.01	-
		8	43-45	inflorescences	0.04-0.10	<0.01	<0.01	<0.01	<0.01
		15	44-47	inflorescences	<0.01-0.01	<0.01	<0.01	<0.01	<0.01
		20-22	49	inflorescences	<0.01	<0.01	<0.01	<0.01	<0.01

1) days after last application
2) at harvest
3) RP 30228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 32490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
* without roots

III. CONCLUSION

Residues of iprodione and its metabolites RP 30228, RP 32490 and RP 32596 declined in broccoli inflorescences during the harvesting period.
In the EU North residues in broccoli inflorescences at the intended PHI of 14 days was 0.01 mg/kg for parent BAS 610 F. Metabolites RP 30228 and RP 32490 were only detected up to 0.02 mg/kg at the beginning of the study. Metabolites RP 32596 and M610F007 were not found above LOQ throughout the study.

Table 6.3.3.1-10: Residues of iprodione in broccoli after three applications of BAS 610 06 F (WG) in Northern Europe

Study details		Crop	Country	Formulation application Rate (kg a.s./ha)[0)]	GS[2] BBCH	DA-LA[1]	Matrix	Residues (mg/kg)				
								iprodione	RP 30228 [3)]	RP 32490 [4)]	RP 32596	M610 F007
								as parent equivalents				
Study code	383567	broccoli	DE	BAS 610 06 F WG 3 x 0.500	42	0	w. pl*.	5.71	0.02	0.02	<0.01	-
DocID:	2011/1120987					8	infl.	0.04	<0.01	<0.01	<0.01	<0.01
Trial No:	L100210					15	infl.	<0.01	<0.01	<0.01	<0.01	<0.01
GLP:	yes					22	infl.	<0.01	<0.01	<0.01	<0.01	<0.01
Year	2010											
Study code:	383567	broccoli	NL	BAS 610 06 F WG 3 x 0.500	43	0	w. pl*.	5.89	<0.01	0.02	<0.01	-
Doc ID:	2011/1120987					8	infl.	0.10	<0.01	<0.01	<0.01	<0.01
Trial No:	L100211					15	infl.	0.01	<0.01	<0.01	<0.01	<0.01
GLP:	yes					20	infl.	<0.01	<0.01	<0.01	<0.01	<0.01
Year:	2010											

1) days after last application
2) at harvest
3) RP 30228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 32490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
infl. inflorescences

Tomato

Table 6.3.3.1-11: cGAP for the use of BAS 610 F in/on tomato

Crop	Maximum applied dose	Water volume	PHI	Application method	Application timing
Tomato	4 x 1.12 kg BAS 610 F/ha	200-2000 L/ha	21	spray application	BBCH 50-89

PHI = pre-harvest interval

Table 6.3.3.1-12: GAP information of residue trials conducted in tomato in 2009-2010 in Northern Europe

Region	Country (No of trials) Year	Formulation	Application[0]				DALA[1]
			Method	Rate[0] (kg a.s./ha)	Spray conc. (kg a.s./hL)	No	
Southern EU	France (1) Italy (1) Spain (1) Greece (1) 2009	BAS 610 06 F (WG)	spray application	1.12	0.22	4	0 1 3-±1 7-±1
	France (1) Italy (1) Spain (1) Greece (1) 2010	BAS 610 06 F (WG)	spray application	1.12	0.22	4	0 1 3-±1 7-±1

Moreno S. (2010b)

Full study reference: Moreno S. (2010b): Study on the residue behaviour of Iprodione in tomato after treatment with BAS 610 06 F under field conditions in South Europe, season 2009
BASF DocID 2010/1007191

Richter M. (2011b)

Full study reference: Richter M. (2011b): Determination of residues of RP 32490 (Reg.No. 5079628), RP 30228 (Reg.No. 5079647) and RP 32596 (Reg.No. 85831) in tomato after treatment with BAS 610 06 F under field conditions in South Europe, season 2009
BASF DocID 2011/1218406

A. MATERIALS

1. Test Material:

Description:	BAS 610 06 F (WG)
Lot/Batch #:	08-160018, 750 g/kg iprodione, nominal
Purity:	Not reported
CAS#:	36734-19-7
Development code:	Not reported
Spiking levels:	0.01, 0.1, 1.0, 2.0 mg/kg

2. Test Commodity:

Crop:	Tomatoes
Type:	Fruiting Vegetables
Variety:	Roma, Marros, Albatro, Beladona
Botanical name:	*Solanum lycopersicum*
Crop parts(s) or processed commodity:	Tomato fruit
Sample size:	≥0.5 kg (≥12 fruits)

B. STUDY DESIGN

1. Test procedure

During the 2009 growing season 4 trials with tomatoes were conducted in different representative growing areas in the Southern EU (France, Italy, Spain, Greece) to determine the residue level of iprodione and its metabolites RP30228 (Reg. No 5079647), RP32490 (Reg. No 5079628), RP32596 (Reg. No 85831) in or on raw agricultural commodities (RAC). Selected samples were also analysed for the recently discovered metabolite M610F007 in a separate study. The analytical details are described in CA 6.3.1/6, the results thereof are also included in this summary.
BAS 610 06 H (750 g/kg iprodione, WG) was foliar applied four times at a rate equivalent to 1.103 kg iprodione/ha in spray volumes of 500 L/ha. The applications were performed at growth stages BBCH 512-81 (1st application), BBCH 516-83 (2nd application), BBCH 518-85 (3rd application and BBCH 708-89 (4th application). Tomato fruits were collected immediately after application (0 DALA), 1 DALA, 3±1 DALA and 7±1 DALA at growth stages BBCH 708-89. Samples were stored frozen at or below -18°C for a maximum of 288 days until analysis.

Table 6.3.3.1-13: Target application rates and timings for tomatoes

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
2009	4	4	F	BAS 610 06 F (WG)	BAS 610 F	1.1	500	39 (±) 1 days 27 (±) 1 days 15 (±) 1 days 3 (±) 1 days before harvest

2. Description of analytical procedures

The specimens were analysed for iprodione (BAS 610 F) with BASF Method No 543/0 (L0022/01), quantifying the parent compound with a limit of quantitation (LOQ) of 0.01 mg/kg. Iprodione was extracted with a mixture of acetone, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned against cyclohexane. The final determination of BAS 610 F was performed by HPLC-MS/MS. Iprodione metabolites RP 30228 (Reg. No 5079647), RP 32490 (Reg. No 5079628), RP 32596 (Reg. No 85831) were analysed with BASF Method No L0180/01, quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. Metabolites were extracted from tomatoes with a mixture of acetonitrile and water formic acid. An aliquot of the extract was centrifuged, diluted with a mixture of acetonitrile, water and formic acid. The final determination of all analytes was performed by UPLC-MS/MS.

Table 6.3.3.1-14: Summary of recoveries of iprodione and its metabolites in/on tomato

Matrix	Fortification Level (mg/kg)	Summary Recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF Method No 543/0		**BAS 610 F**					
Tomato fruit	0.01, 0.10, 1.0, 2.0	11	89	7.3			
BASF Method No L0180/01		**RP 32490 (Reg. No 5079628)**			**RP 32596 (Reg. No 85831)**		
Tomato fruit	0.01, 0.10	8	89.7	14	8	84.8	12
BASF Method No L0180/01		**RP 30228 (Reg. No 5079647)**					
Tomato fruit	0.01, 0.10	8	89.2	8.1			

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.3.1-15, detailed residue levels are shown in Table 6.3.3.1-16.

Residues of BAS 610 F ranged from 0.15 to 1.10 mg/kg at the beginning of the study declining to values between 0.17 and 1.10 mg/kg at the intended PHI of 3 days. Metabolite RP 30228 was found at day 0 and after one day at or slightly above LOQ, but at the PHI no quantifiable residues were detected. Residues of metabolite RP 32490 were found at all times at comparable level. At the PHI they ranged between 0.012 and 0.034 mg/kg. Residues of metabolites RP 32596 and M610F007 were below the LOQ of the analytical method (<0.01 mg/kg) in all samples and at all harvest times.

No residues at or above the LOQ (0.01 mg/kg) were found in any control sample.

Table 6.3.3.1-15: Summary of residues in tomato

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Matrix	Range of residues (mg/kg)				
					iprodione	RP 30228 3)	RP 32490 4)	RP 32596	M610 F007
					as parent equivalents				
EU South	2009	0	708-89	fruit	0.15-1.10	<0.01-0.01	0.015-0.035	<0.01	-
		1	708-89	fruit	0.13-1.20	<0.01-0.012	0.013-0.034	<0.01	<0.01
		3±1	708-89	fruit	0.17-1.10	<0.01	0.012-0.034	<0.01	<0.01
		7±1	708-89	fruit	0.13-0.79	<0.01	0.011-0.037	<0.01	<0.01

1) days after last application
2) at harvest
3) RP 30228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 32490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146

III. CONCLUSION

In tomato, metabolite RP 32490 was found at all sampling times. At the intended harvest time (PHI 3±1 days) residues of BAS 610 F and RP 32490 in tomato fruit ranged from 0.17-1.10 mg/kg and from 0.012-0.034 mg/kg, respectively. Residues of metabolites RP 30228, RP 32596 and M610F007 were below the LOQ of the analytical method (<0.01 mg/kg) in all samples.

Table 6.3.3.1-16: Residues of iprodione in/on tomatoes after four applications of BAS 610 06 F (WG) in Southern Europe

Study details		Crop	Country	Formulation application Rate (kg a.s./ha)[0)]	GS[2] BBCH	DA-LA[1]	Matrix	Residues (mg/kg)				
								iprodione	RP 30228 [3)]	RP 32490 [4)]	RP 32596	M610 F007
								as parent equivalents				
Study code	357851	tomato	France	BAS 610 06 F 4 x 1.100	708-82	0	fruit	1.10	<0.01	0.015	<0.01	-
Doc ID:	2010/1007191					1	fruit	0.95	<0.01	0.019	<0.01	<0.01
	2011/1218406					3	fruit	0.22	<0.01	0.012	<0.01	<0.01
Trial No:	L090293					7	fruit	0.33	<0.01	0.013	<0.01	<0.01
GLP:	yes											
Year:	2009											
Study code	357851	tomato	Italy	BAS 610 06 F 4 x 1.100	87-89	0	fruit	1.00	0.01	0.025	<0.01	-
Doc ID:	2010/1007191					1	fruit	1.20	0.012	0.023	<0.01	<0.01
	2011/1218406					4	fruit	1.10	0.012	0.028	<0.01	<0.01
Trial No:	L090294					8	fruit	0.16	<0.01	0.022	<0.01	<0.01
GLP:	yes											
Year:	2009											
Study code	357851	tomato	Spain	BAS 610 06 F 4 x 1.100	77-79	0	fruit	1.10	<0.01	0.025	<0.01	-
Doc ID:	2010/1007191					1	fruit	0.90	<0.01	0.013	<0.01	<0.01
	2011/1218406					3	fruit	1.00	<0.01	0.012	<0.01	<0.01
Trial No:	L090295					7	fruit	0.79	<0.01	0.011	<0.01	<0.01
GLP:	Yes											
Year:	2009											
Study code	357851	tomato	Greece	BAS 610 06 F 4 x 1.100	75-82	0	fruit	0.15	<0.01	0.035	<0.01	-
Doc ID:	2010/1007191					1	fruit	0.13	<0.01	0.034	<0.01	<0.01
	2011/1218406					3	fruit	0.17	<0.01	0.036	<0.01	<0.01
Trial No:	L090296					7	fruit	0.13	0.011	0.037	<0.01	<0.01
GLP:	yes											
Year:	2009											

1) days after last application
2) at harvest
3) RP 30228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 32490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146

Moreno S. (2012b)

Full study reference: Moreno S. (2012b): Study on the residue behaviour of Iprodione in tomato after treatment with BAS 610 06 F under field conditions in South Europe, season 2010
BASF DocID 2011/1120983

A. MATERIALS

1. Test Material:

Description:	BAS 610 06 F (WG)
Lot/Batch #:	08-160018, 750 g/kg iprodione, nominal
Purity:	Not reported
CAS#:	36734-19-7
Development code:	Not reported
Spiking levels:	0.01, 0.1, 1.0, 10.0 mg/kg

2. Test Commodity:

Crop:	Tomatoes
Type:	Fruiting Vegetables
Variety:	Roma, Marros, Albatro, Beladona
Botanical name:	*Solanum lycopersicum*
Crop parts(s) or processed commodity:	Tomato fruit
Sample size:	≥0.870 kg (≥12 fruits)

B. STUDY DESIGN

1. Test procedure

During the 2010 growing season 4 trials with tomatoes were conducted in different representative growing areas in the Southern EU (France, Italy, Spain, Greece) to determine the residue level of iprodione and its metabolites RP30228 (Reg. No 5079647), RP32490 (Reg. No 5079628), RP32596 (Reg. No 85831) in or on raw agricultural commodities (RAC). Selected samples were also analysed for the recently discovered metabolite M610F007 in a separate study. The analytical details are described in CA 6.3.1/6, the results thereof are also included in this summary.
BAS 610 06 H (750 g/kg iprodione, WG) was foliar applied four times at a rate equivalent to 1.103 kg iprodione/ha in spray volumes of 500 L/ha. The applications were performed at growth stages BBCH 54-79 (1st application), BBCH 55-85 (2nd and 3rd application) and BBCH 71-89 (4th application). Tomato fruits were collected immediately after application (0 DALA), 1 DALA, 3 DALA and 6-7 DALA at growth stages BBCH 71-89. Samples were stored frozen at or below -18°C for a maximum of 419 days until analysis.

Table 6.3.3.1-17: Target application rates and timings for tomato

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
2010	4	4	F	BAS 610 06 F (WG)	BAS 610 F	1.1	500	39 (±) 1 days 27 (±) 1 days 15 (±) 1 days 3 (±) 1 days before harvest

2. Description of analytical procedures

The specimens were analysed for iprodione (BAS 610 F) and its metabolites RP 30228 (Reg. No 5079647), RP 32490 (Reg. No 5079628), RP 32596 (Reg. No 85831) with BASF Method No L0180/01, quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. Iprodione (BAS 610 F) and its metabolites were extracted with a mixture of acetonitrile and water. An aliquot of the extract was centrifuged and diluted with a mixture of acetonitrile, water and formic acid. The final determination of BAS 610 F was performed by UPLC-MS/MS.

Table 6.3.3.1-18: Summary of recoveries of iprodione and its metabolites in/on tomato

Matrix	Fortification Level (mg/kg)	Summary Recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF Method No L0180/01		**BAS 610 F**			**RP 30228 (Reg. No 5079647)**		
Tomato fruit	0.01, 0.10, 1.0, 10.0	4	99.7	9.9	4	98.5	7.9
BASF Method No L0180/01		**RP 32490 (Reg. No 5079628)**			**RP 32596 (Reg. No 85831)**		
Tomato fruit	0.01, 0.10, 1.0, 10.0	4	85.6	8.5	4	95.6	9.8

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.3.1-19, detailed residue levels are shown in Table 6.3.3.1-20.

Residues of BAS 610 F ranged from 0.37 to 1.37 mg/kg at the beginning of the study declining to values between 0.17 and 1.61 mg/kg at the intended PHI of 3 days. . Residues of metabolite RP 32490 were found at all times at comparable level. At the PHI they ranged between 0.03 and 0.04 mg/kg. Residues of metabolites RP 30228, RP 32596 and M610F007 were below the LOQ of the analytical method (<0.01 mg/kg) in all samples and at all harvest times.

No residues at or above the LOQ (0.01 mg/kg) were found in any control sample.

Table 6.3.3.1-19: Summary of residues in tomato

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)					
				Matrix	iprodione	RP 30228 [3)]	RP 32490 [4)]	RP 32596	M610 F007
					as parent equivalents				
EU South	2010	0	71-89	fruit	0.37-1.37	<0.01	0.03-0.04	<0.01	-
		1	74-89	fruit	0.18-1.69	<0.01	0.03-0.04	<0.01	<0.01
		3	74-89	fruit	0.17-1.61	<0.01	0.03-0.04	<0.01	<0.01
		6-7	85-89	fruit	0.05-1.88	<0.01	0.01-0.05	<0.01	<0.01

1) days after last application
2) at harvest
3) RP 30228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 32490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146

III. CONCLUSION

In tomato, metabolite RP 32490 was found at all sampling times above LOQ. At the intended harvest time (PHI 3 days) residues of BAS 610 F and RP 32490 ranged from 0.17-1.61 mg/kg and from 0.03-0.04 mg/kg, respectively. Residues of metabolites RP 30228, RP 32596 and M610F007 were below the LOQ of the analytical method (<0.01 mg/kg) in all samples.

Table 6.3.3.1-20: Residues of iprodione in/on tomato after four applications of BAS 610 06 F (WG) in Southern Europe

Study details		Crop	Country	Formulation application Rate (kg a.s./ha)[0)]	GS[2] BBCH	DA-LA[1]	Matrix	Residues (mg/kg)				
								iprodione	RP 30228 [3)]	RP 32490 [4)]	RP 32596	M610 F007
								as parent equivalents				
Study code	357849	tomato	France	BAS 610 06 F 4 x 1.100	74-85	0	fruit	0.48	<0.01	0.04	<0.01	-
Doc ID:	2011/1120983					1	fruit	0.81	<0.01	0.04	<0.01	<0.01
Trial No:	L100199					3	fruit	0.45	<0.01	0.03	<0.01	<0.01
GLP:	yes					7	fruit	0.05	<0.01	0.01	<0.01	<0.01
Year:	2010											
Study code	357849	tomato	Italy	BAS 610 06 F 4 x 1.100	87-89	0	fruit	1.35	<0.01	0.03	<0.01	-
Doc ID:	2011/1120983					1	fruit	1.69	<0.01	0.04	<0.01	<0.01
Trial No:	L100200					3	fruit	1.47	<0.01	0.04	<0.01	<0.01
GLP:	yes					7	fruit	1.88	<0.01	0.05	<0.01	<0.01
Year:	2010											
Study code	357849	tomato	Spain	BAS 610 06 F 4 x 1.100	83-89	0	fruit	1.37	<0.01	0.03	<0.01	-
Doc ID:	2011/1120983					1	fruit	1.68	<0.01	0.03	<0.01	<0.01
Trial No:	L100201					3	fruit	161	<0.01	0.04	<0.01	<0.01
GLP:	yes					7	fruit	1.31	<0.01	0.03	<0.01	<0.01
Year:	2010											
Study code	357849	tomato	Greece	BAS 610 06 F 4 x 1.100	71-89	0	fruit	0.37	<0.01	0.03	<0.01	-
Doc ID:	2011/1120983					1	fruit	0.18	<0.01	0.03	<0.01	<0.01
Trial No:	L100202					3	fruit	0.17	<0.01	0.04	<0.01	<0.01
GLP:	yes					6	fruit	0.20	<0.01	0.04	<0.01	<0.01
Year:	2010											

1) days after last application
2) at harvest
3) RP 30228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 32490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146

Plum

Table 6.3.3.1-21: cGAP for the use of BAS 610 F in/on Plum

Crop	Maximum applied dose	Water volume	PHI	Application method	Application timing
Plum	2 x 0.750 kg BAS 610 F/ha	300-1500 L/ha	14	spray application	BBCH 55-69 BBCH 81-87

PHI = pre-harvest interval

Table 6.3.3.1-22: GAP information of residue trials conducted in plum in 2009-2010 in Northern Europe

Region	Country (No of trials) Year	Formulation	Application[0]				DALA[1]
			Method	Rate[0] (kg a.s./ha)	Spray conc. (kg a.s./hL)	No	
Northern EU	Germany (1) Belgium (1) Netherlands (1) France (1) 2009	BAS 610 06 F (WG)	spray application	0.750	0.08	2	0 7-±1 14-±1 21-±1
	Germany (1) Belgium (1) Denmark (1) France (1) 2010	BAS 610 06 F (WG)	spray application	0.750	0.08	2	0 7-±1 14-±1 21-±1
Southern EU	France (1) Italy (1) Spain (1) Greece (1) 2009	BAS 610 06 F (WG)	spray application	0.750	0.08	2	0 7-±1 14-±1 21-±1
	France (1) Italy (1) Greece (1) 2010	BAS 610 06 F (WG)	spray application	0.750	0.08	2	0 7-±1 14-±1 21-±1

Moreno S. (2010c)

Full study reference: Moreno S. (2010c): Study on the residue behaviour of Iprodione in plums after treatment with BAS 610 06 F under field conditions in North and South Europe, season 2009
BASF DocID 2010/1007193

Richter M. (2011c)

Full study reference: Richter M. (2011c): Determination of residues of RP 32490 (Reg.No. 5079628), RP 30228 (Reg.No. 5079647) and RP 32596 (Reg.No. 85831) in plums after treatment with BAS 610 06 F under field conditions in North and South Europe, season 2009
BASF DocID 2011/1249468

A. MATERIALS

1. Test Material:

Description:	BAS 610 06 F (WG)
Lot/Batch #:	08-160018, 750 g/kg iprodione, nominal
Purity:	Not reported
CAS#:	36734-19-7
Development code:	Not reported
Spiking levels:	0.01, 0.1, 1.0 mg/kg

2. Test Commodity:

Crop:	Plums
Type:	Stone fruits
Variety:	Elena, Ton, Cotan, Helena, Ente 626, Angelino, Flavour King
Botanical name:	*Prunus domestica*
Crop parts(s) or processed commodity:	Plum fruit
Sample size:	≥1.1 kg (≥24 fruits)

B. STUDY DESIGN

1. Test procedure

During the 2009 growing season 7 trials with plums were conducted in different representative growing areas in the EU, 4 in the North (Germany, France, Netherlands, Belgium) and 3 in the South (Italy, Greece, France) to determine the residue level of iprodione and its metabolites RP30228 (Reg. No 5079647), RP32490 (Reg. No 5079628), RP32596 (Reg. No 85831) in or on raw agricultural commodities (RAC). BAS 610 06 H (750 g/kg iprodione, WG) was applied twice at a rate equivalent to 0.750 kg iprodione/ha in spray volumes of 1000 L/ha. The applications were performed at growth stages BBCH 75-81 (1st application) and BBCH 77-85 (2nd application). Fruits were collected immediately after application (BBCH 77-85), 6-8 days (BBCH 78-87), 13-15 days (BBCH 85-89) and 20-21 days (BBCH 87-89) after the last application. Samples were stored frozen at or below -18°C for a maximum of 277 days until analysis.

Table 6.3.3.1-23: Target application rates and timings for plum

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
2009	7	2	F	BAS 610 06 F (WG)	BAS 610 F	0.750	1000	28 (±) days 14 (±) days before harvest

2. Description of analytical procedures

The specimens were analysed for iprodione (BAS 610 F) with BASF Method No 543/0, quantifying the parent compound with a limit of quantitation (LOQ) of 0.01 mg/kg. Iprodione (BAS 610 F) was extracted with a mixture of acetone, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned against cyclohexane. The final determination of BAS 610 F was performed by HPLC-MS/MS. The metabolites RP 30228 (Reg. No 5079647), RP 32490 (Reg. No 5079628), RP 32596 (Reg. No 85831) were analysed with BASF Method No L0180/01, quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. The metabolites were extracted from plums with a mixture of acetonitrile, water and formic acid. An aliquot of the extract was centrifuged and measured directly. The final determination of all analytes was performed by UPLC-MS/MS.

Table 6.3.3.1-24: Summary of recoveries of iprodione and its metabolites in/on plums

Matrix	Fortification Level (mg/kg)	Summary Recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF Method No 543/0		**BAS 610 F**					
Plum fruit	0.01, 0.10, 1.0	9	99.3	12			
BASF Method No L0180/01		**RP 32490 (Reg. No 5079628)**			**RP 32596 (Reg. No 85831)**		
Whole plant*	0.01, 0.10, 10	12	89.7	12	12	81.2	7.5
BASF Method No L0180/01		**RP 30228 (Reg. No 5079647)**					
Whole plant*	0.01, 0.10	12	92.7	10			

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.3.1-25, detailed residue levels are shown in Table 6.3.3.1-26 and Table 6.3.3.1-27.

Residues of iprodione in/on plum fruit declined during the harvesting period and were slightly higher in the EU North, compared to residues in the EU South, at all harvest times.

In the EU North residues of BAS 610 F ranged from 0.20 to 1.1 mg/kg at the beginning of the study and degraded to values between 0.14 and 0.62 mg/kg at the intended PHI of 14 days. Residues of metabolite RP 32490 were found in three out of seven trials up to 0.015 mg/kg. In all other trials no residues above LOQ could be seen. Also, residues of metabolites RP 30228 and RP 32596 were below the LOQ of the analytical method (<0.01 mg/kg) in all samples and at all harvest times

In the EU South residues of BAS 610 F ranged from 0.1 to 0.31 mg/kg at day 0 and decreased to values between 0.067 and 0.16 mg/kg at the PHI of 14 days. None of the metabolites was detected above the LOQ of the analytical method (<0.01 mg/kg) throughout the study.

No residues at or above the LOQ (0.01 mg/kg) were found in any control sample.

Table 6.3.3.1-25: Summary of residues in plum

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)				
				Matrix	iprodione	RP 30228 [3]	RP 32490 [4]	RP 32596
					as parent equivalents			
EU North	2009	0	85	fruit	0.20-1.1	<0.01	<0.01	<0.01
		6-8	85-87	fruit	0.21-0.85	<0.01	<0.01-0.011	<0.01
		13-15	85-87	fruit	**0.14-0.62**	**<0.01**	**<0.01-0.015**	**<0.01**
		20-21	87-89	fruit	0.13-0.46	<0.01	<0.01-0.012	<0.01
EU South	2009	0	77-85	fruit	0.1-0.31	<0.01	<0.01	<0.01
		7	78-87	fruit	0.090-0.31	<0.01	<0.01	<0.01
		14	85-89	fruit	**0.067-0.16**	**<0.01**	**<0.01**	**<0.01**
		20-21	87-89	fruit	0.041-0.14	<0.01	<0.01	<0.01

1) days after last application
2) at harvest
3) RP 30228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 32490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

Residues of iprodione and its metabolite RP 032490 declined in/on plums during the harvesting period and were slightly higher in trials performed in the EU North compared to trials in the South.
In the EU residues of BAS 610 F ranged from 0.1-1.1 mg/kg, 0.09-0.85 mg/kg, 0.067-0.62 mg/kg (PHI) and 0.041-0.46 mg/kg at 0, 6-8, 13-15 (PHI), and 20-21 DALA, respectively. Residues of metabolite RP 032490 were <0.01 mg/kg, and ranged from <0.01-0.011 mg/kg, <0.01-0.015 mg/kg (PHI) and <0.01-0.012 mg/kg at 0, 6-8, 13-15 and 20-21 DALA, respectively. Residues of metabolites RP 030228 and RP 032596 were below the LOQ of the analytical method (<0.01 mg/kg) in all samples and at all harvest times.

Table 6.3.3.1-26: Residues of iprodione in/on plums after two applications of BAS 610 06 F (WG) in Northern Europe

Study details		Crop	Country	Formulation application Rate (kg a.s./ha)[0)]	GS[2] BBCH	DA-LA[1]	Matrix	Residues (mg/kg)			
								ipro-dione	RP 30228 [3)]	RP 32490 [4)]	RP 32596
								as parent equivalents			
Study code	357848	plum	DE	BAS 610 06 F WG 2 x 0.750	85	0	fruit	0.40	<0.01	<0.01	<0.01
Doc ID:	2010/1007193					8	fruit	0.21	<0.01	<0.01	<0.01
	2011/1249468					15	fruit	0.26	<0.01	<0.01	<0.01
Trial No:	L090286					21	fruit	0.26	<0.01	<0.01	<0.01
GLP:	yes										
Year:	2009										
Study code	357848	plum	BE	BAS 610 06 F WG 2 x 0.750	85	0	fruit	0.22	<0.01	<0.01	<0.01
Doc ID:	2010/1007193					6	fruit	0.21	<0.01	0.011	<0.01
	2011/1249468					13	fruit	0.22	<0.01	0.015	<0.01
Trial No:	L090287					20	fruit	0.14	<0.01	<0.01	<0.01
GLP:	yes										
Year:	2009										
Study code	357848	plum	NL	BAS 610 06 F WG 2 x 0.750	85	0	fruit	0.20	<0.01	<0.01	<0.01
Doc ID:	2010/1007193					6	fruit	0.22	<0.01	0.011	<0.01
	2011/1249468					13	fruit	0.14	<0.01	<0.01	<0.01
Trial No:	L090288					20	fruit	0.13	<0.01	0.012	<0.01
GLP:	yes										
Year:	2009										
Study code	357848	plum	FR	BAS 610 06 F WG 2 x 0.750	85	0	fruit	1.1	<0.01	<0.01	<0.01
Doc ID:	2010/1007193					6	fruit	0.85	<0.01	<0.01	<0.01
	2011/1249468					13	fruit	0.62	<0.01	<0.01	<0.01
Trial No:	L090289					20	fruit	0.46	<0.01	0.011	<0.01
GLP:	yes										
Year:	2009										

1) days after last application
2) at harvest
3) RP 030228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 032490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146

Table 6.3.3.1-27: Residues of iprodione in/on plums after two applications of BAS 610 06 F (WG) in Southern Europe

Study details		Crop	Country	Formulation application Rate (kg a.s./ha)[0)]	GS[2] BBCH	DA-LA[1]	Matrix	Residues (mg/kg)			
								iprodione	RP 30228 [3)]	RP 32490 [4)]	RP 32596
								as parent equivalents			
Study code	357848	plum	FR	BAS 610 06 F WG 2 x 0.750	77	0	fruit	0.16	<0.01	<0.01	<0.01
Doc ID:	2010/1007193					7	fruit	0.13	<0.01	<0.01	<0.01
	2011/1249468					14	fruit	0.092	<0.01	<0.01	<0.01
Trial No:	L090290					21	fruit	0.041	<0.01	<0.01	<0.01
GLP:	yes										
Year:	2009										
Study code	357848	plum	GR	BAS 610 06 F WG 2 x 0.750	79-85	0	fruit	0.31	<0.01	<0.01	<0.01
Doc ID:	2010/1007193					7	fruit	0.31	<0.01	<0.01	<0.01
	2011/1249468					14	fruit	0.16	<0.01	<0.01	<0.01
Trial No:	L090291					20	fruit	0.14	<0.01	<0.01	<0.01
GLP:	yes										
Year:	2009										
Study code	357848	plum	IT	BAS 610 06 F WG 2 x 0.750	81	0	fruit	0.10	<0.01	<0.01	<0.01
Doc ID:	2010/1007193					7	fruit	0.090	<0.01	<0.01	<0.01
	2011/1249468					14	fruit	0.067	<0.01	<0.01	<0.01
Trial No:	L090292					20	fruit	0.080	<0.01	<0.01	<0.01
GLP:	yes										
Year:	2009										

1) days after last application
2) at harvest
3) RP 30228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 32490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146

Moreno S. (2012c)

Full study reference: Moreno S. (2012c): Study on the residue behaviour of Iprodione in plums after treatment with BAS 610 06 F under field conditions in North and South Europe, season 2010
BASF DocID 2011/1120982

A. MATERIALS

1. Test Material:

Description:	BAS 610 06 F (WG)
Lot/Batch #:	08-160018, 750 g/kg iprodione, nominal
Purity:	Not reported
CAS#:	36734-19-7
Development code:	Not reported
Spiking levels:	0.01, 0.1, 1.0 mg/kg

2. Test Commodity:

Crop:	Plums
Type:	Stone fruits
Variety:	Hauszwetsche, Sultan, Jubileum, Quetsche, Ente 626, Antzelino, Angeleno
Botanical name:	*Prunus domestica*
Crop parts(s) or processed commodity:	Plum fruit
Sample size:	≥1.0 kg (≥24 fruits)

B. STUDY DESIGN

1. Test procedure

During the 2010 growing season 7 trials with plums were conducted in different representative growing areas in the EU, 4 in the North (Germany, France, Denmark, Belgium) and 3 in the South (Italy, Greece, France) to determine the residue level of iprodione and its metabolites RP30228 (Reg. No 5079647), specimens RP32490 (Reg. No 5079628), RP32596 (Reg. No 85831) in or on raw agricultural commodities (RAC). BAS 610 06 H (750 g/kg iprodione, WG) was applied twice at a rate equivalent to 0.750 kg iprodione/ha in spray volumes of 1,000 L/ha. The applications were performed at growth stages BBCH 73-83 (1st application) and BBCH 77-85 (2nd application). Fruits were collected immediately after application (BBCH 77-85), 6-8 days (BBCH 79-85), 13-15 days (BBCH 85-89) and 20-21 days (BBCH 87-89) after the last application. Samples were stored frozen at or below -18°C for a maximum of 446 days until analysis.

Table 6.3.3.1-28: Target application rates and timings for plums

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
2010	7	2	F	BAS 610 06 F (WG)	BAS 610 F	0.750	1000	28 (±) days 14 (±) days before harvest

2. Description of analytical procedures

The specimens were analysed for iprodione (BAS 610 F) and its metabolites RP 30228 (Reg. No 5079647), RP 32490 (Reg. No 5079628) and RP 32596 (Reg. No 85831) with BASF Method No L0180/01, quantifying each relevant analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. Residues were extracted from plums with a mixture of acetonitrile and water. An aliquot of the extract was centrifuged and diluted with a mixture of acetonitrile, water and formic acid. The final determination of all analytes was performed by UPLC-MS/MS.

Table 6.3.3.1-29: Summary of recoveries of iprodione and its metabolites in/on plums

Matrix	Fortification Level (mg/kg)	Summary Recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF Method No L0180/01		**BAS 610 F**			**RP 30228 (Reg. No 5079647)**		
Fruits	0.01, 0.10, 1.0, 10, 40	15	102.7	4.4	15	100.6	6.1
BASF Method No L0180/01		**RP 32490 (Reg. No 5079628)**			**RP 32596 (Reg. No 85831)**		
Fruits	0.01, 0.10, 10	15	94.8	9.8	3	87.9	8.9

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.3.1-30, detailed residue levels are shown in Table 6.3.3.1-31 and Table 6.3.3.1-32.

Residues of iprodione and its metabolites in/on plum fruit were comparable in the EU North and South, at the different harvest times.

In the EU North residues of BAS 610 F ranged from 0.18-0.69 mg/kg at day 0. At the intended PHI of 14 days, the parent compound was found between 0.07 and 0.36 mg/kg. Residues of metabolite RP 32490 were only found in one trial up to 0.02 mg/kg. Residues of metabolites RP 30228 and RP 32596 were below the LOQ of the analytical method (<0.01 mg/kg) in all samples and at all harvest times.

In the EU South residues of BAS 610 F ranged from 0.20-0.55 mg/kg in the beginning. They degraded to values between 0.09 and 0.28 mg/kg at the PHI of 14 days. In the French trial, both RP 30228 and RP 32490 were found lightly above LOQ (0.01 and 0.02 mg/kg, respectively), but in all other trials no residues above LOQ were detected. Residues of metabolites RP 32596 were below the LOQ of the analytical method (<0.01 mg/kg) in all samples and at all harvest times.

No residues at or above the LOQ (0.01 mg/kg) were found in any control sample.

Table 6.3.3.1-30: Summary of residues in plum

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Matrix	Range of residues (mg/kg)			
					iprodione	RP 30228 [3]	RP 32490 [4]	RP 32596
					as parent equivalents			
EU North	2010	0	81-85	fruit	0.18-0.69	<0.01	<0.01	<0.01
		6-8	85	fruit	0.07-0.23	<0.01	<0.01	<0.01
		13-15	87-89	fruit	**0.07-0.36**	**<0.01**	**<0.01-0.01**	**<0.01**
		20-21	89	fruit	0.09-0.26	<0.01	<0.01-0.02	<0.01
EU South	2010	0	77-81	fruit	0.20-0.55	<0.01	<0.01	<0.01
		6-7	79-84	fruit	0.12-0.58	<0.01	<0.01-0.01	<0.01
		14-15	85-89	fruit	**0.09-0.28**	**<0.01-0.01**	**<0.01**	**<0.01**
		20-21	87-89	fruit	0.11-0.64	<0.01-0.02	<0.01-0.02	<0.01

1) days after last application
2) at harvest
3) RP 30228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 32490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

Residues of iprodione and its metabolites in/on plum fruit were comparable in the EU North and South, at the different harvest times.
Residues of iprodione parent were found to range between 0.07 and 0.36 mg/kg in the North and between 0.09 and 0.28 mg/kg in the South at the intended PHI of 14 days.
Metabolite RP 32490 was formed during the study in amounts up to 0.02 mg/kg in both regions.
Metabolite RP30228 was found at LOQ at some sampling times in the North. At the PHI no residues at or below the LOQ were seen.
Residues of metabolite RP 32596 were below the LOQ of the analytical method (<0.01 mg/kg) in all samples and at all harvest times.

Table 6.3.3.1-31: Residues of iprodione in/on plums after two applications of BAS 610 06 F (WG) in Northern Europe

Study details		Crop	Country	Formulation application Rate (kg a.s./ha)[0)]	GS[2] BBCH	DA-LA[1]	Matrix	Residues (mg/kg)			
								iprodione	RP 30228 [3)]	RP 32490 [4)]	RP 32596
								as parent equivalents			
Study code	357845	plums	DE	BAS 610 06 F WG 2 x 0.750	85	0	fruit	0.18	<0.01	<0.01	<0.01
Doc ID:	2011/1120982					6	fruit	0.16	<0.01	<0.01	<0.01
Trial No:	L100203					13	fruit	0.15	<0.01	<0.01	<0.01
GLP:	yes					20	fruit	0.16	<0.01	<0.01	<0.01
Year:	2010										
Study code	357845	plums	BE	BAS 610 06 F WG 2 x 0.750	83	0	fruit	0.25	<0.01	<0.01	<0.01
Doc ID:	2011/1120982					7	fruit	0.16	<0.01	<0.01	<0.01
Trial No:	L100204					14	fruit	0.07	<0.01	<0.01	<0.01
GLP:	yes					21	fruit	0.16	<0.01	<0.01	<0.01
Year:	2010										
Study code	357845	plums	DK	BAS 610 06 F WG 2 x 0.750	81	0	fruit	0.23	<0.01	<0.01	<0.01
Doc ID:	2011/1120982					8	fruit	0.07	<0.01	<0.01	<0.01
Trial No:	L100205					15	fruit	0.07	<0.01	<0.01	<0.01
GLP:	yes					22	fruit	0.09	<0.01	<0.01	<0.01
Year:	2010										
Study code	357845	plums	FR	BAS 610 06 F WG 2 x 0.750	81	0	fruit	0.69	<0.01	<0.01	<0.01
Doc ID:	2011/1120982					7	fruit	0.23	<0.01	<0.01	<0.01
Trial No:	L100206					14	fruit	0.36	<0.01	0.01	<0.01
GLP:	yes					21	fruit	0.26	<0.01	0.02	<0.01
Year:	2010										

1) days after last application
2) at harvest
3) RP 030228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 032490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146

Table 6.3.3.1-32: Residues of iprodione in/on plums after two applications of BAS 610 06 F (WG) in Southern Europe

Study details		Crop	Country	Formulation application Rate (kg a.s./ha)[0)]	GS[2] BBCH	DA-LA[1]	Matrix	Residues (mg/kg)			
								ipro-dione	RP 30228 [3)]	RP 32490 [4)]	RP 32596
								as parent equivalents			
Study code	357845	plums	FR	BAS 610 06 F WG 2 x 0.750	77	0	fruit	0.55	<0.01	<0.01	<0.01
Doc ID:	2011/1120982					7	fruit	0.58	<0.01	0.01	<0.01
Trial No:	L100207					15	fruit	0.28	0.01	<0.01	<0.01
GLP:	yes					21	fruit	0.64	0.02	<0.01	<0.01
Year:	2010										
Study code	357845	plums	GR	BAS 610 06 F WG 2 x 0.750	78	0	fruit	0.48	<0.01	<0.01	<0.01
Doc ID:	2011/1120982					6	fruit	0.22	<0.01	<0.01	<0.01
Trial No:	L100208					14	fruit	0.24	<0.01	<0.01	<0.01
GLP:	yes					20	fruit	0.22	<0.01	<0.01	<0.01
Year:	2010										
Study code	357845	plums	IT	BAS 610 06 F WG 2 x 0.750	79-81	0	fruit	0.20	<0.01	<0.01	<0.01
Doc ID:	2011/1120982					7	fruit	0.12	<0.01	<0.01	<0.01
Trial No:	L100209					15	fruit	0.09	<0.01	<0.01	<0.01
GLP:	yes					21	fruit	0.11	<0.01	<0.01	<0.01
Year:	2010										

1) days after last application
2) at harvest
3) RP 30228 (Reg. No 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 32490 (Reg. No 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146

CA 6.3.3.2 Summary of metabolite residue data and conclusions thereof

RP30228 and RP32490

In the supervised field trials reported in this chapter, the metabolites RP30228 and RP32490 were analysed separately in order to assess their relevance. The following table shows in how many samples residues above the limit of quantitation were found:

Table 6.3.3.2-1: Number of samples analysed for RP30228 and RP32490

Crop	No. of samples analysed	No. of samples with residues above LOQ	
		RP30228	RP32490
carrot	112	91	52
lettuce	96	67	74
broccoli	16	2	1
tomato	32	3	32
plum	56	2	8

The residues for RP30228 and RP32490 found are summarized in Table 6.3.3.2-2. Apart from the normal description in mg/kg they are also given as percent of parent residue:

From the results obtained from specifically analyzing the metabolites RP30228 and RP32490 the following conclusions can be drawn:

- In approximately half of the samples analysed both metabolites could be quantified (residues above LOQ)
- In accordance with findings from metabolism studies, the amounts of both analytes vary considerably, within one crop they are relatively uniform
- Calculating the mean values of metabolite residues expressed as percentage of parent lead to following overall mean: on average RP30228 amount to 6% and RP32490 to 5% of the respective parent residue.
- Calculating a mean value for both metabolites without day 0 results (which can be regarded as untypical regarding metabolite distribution) leads to 8% for RP30228 and 6% for RP32490

Due to the inhomogeneous distribution of residues of RP30228 and RP32490 found in five crops investigated the values obtained can only be regarded as rough indicators for metabolite relevance. However, despite this inaccuracy it seems necessary to take both metabolites into account for risk assessment. In good approximation, it is therefore proposed to assume that both

metabolites RP30228 and RP32490 are formed in 10% of parent iprodione.

Based on the data shown below these values are used for the consumer risk assessment of iprodione as well as for the exposure calculation for the metabolites in connection with the TTC concept (see chapter CA 6.7).

Table 6.3.3.2-2: Residue results of RP30228 and RP32490

Report No	Crop	Commodity	DALA	RP30228		RP32490	
				mg/kg	% of parent	mg/kg	% of parent
CA 6.3.1/4	carrot	whole plant	0	0.02	0.2%	0.01	0.1%
		roots	22	<0.01	-	<0.01	-
		tops	22	0.08	1%	0.09	1%
		roots	28	<0.01	-	<0.01	-
		tops	28	0.08	1%	0.07	1%
		roots	35	<0.01	-	<0.01	-
		tops	35	0.10	2%	0.08	1%
		whole plant	0	0.05	1%	<0.01	-
		roots	20	0.02	14%	<0.01	-
		tops	20	0.21	10%	0.02	1%
		roots	28	0.01	8%	<0.01	-
		tops	28	0.16	10%	0.01	1%
		roots	35	0.01	8%	<0.01	-
		tops	35	0.08	10%	<0.01	-
		whole plant	0	0.03	1%	<0.01	-
		roots	22	<0.01	-	<0.01	-
		tops	22	0.06	2%	0.02	1%
		roots	26	<0.01	-	<0.01	-
		tops	26	0.12	3%	0.03	1%
		roots	35	<0.01	-	<0.01	-
		tops	35	0.10	4%	0.02	1%
		whole plant	0	0.01	0.2%	0.02	0.4%
		roots	21	<0.01	-	<0.01	-
		tops	21	0.20	5%	0.10	2%
		roots	28	<0.01	-	<0.01	-
		tops	28	0.20	4%	0.08	1%
		roots	35	<0.01	-	<0.01	-
		tops	35	0.33	5%	0.11	2%
		whole plant	0	0.03	0.4%	0.02	0.2%
		roots	21	0.01	4%	<0.01	-
		tops	21	0.05	1%	0.08	2%
		roots	27	0.02	6%	<0.01	-
		tops	27	0.08	2%	0.07	2%
		roots	35	0.01	7%	<0.01	-
		tops	35	0.06	3%	0.04	2%
		whole plant	0	0.10	0.3%	0.08	0.2%
		roots	21	0.02	6%	<0.01	-
		tops	21	0.17	2%	0.10	1%
		roots	27	0.02	7%	<0.01	-
		tops	27	0.17	2%	0.08	1%
		roots	35	0.03	9%	<0.01	-
		tops	35	0.19	2%	0.07	1%
		whole plant	0	0.12	1%	0.02	0.2%
		roots	21	0.03	10%	<0.01	-
		tops	21	0.20	4%	0.05	1%
		roots	27	0.04	11%	<0.01	-
		tops	27	0.16	3%	0.04	1%
		roots	35	0.04	11%	<0.01	-
		tops	35	0.18	4%	0.04	1%

Report No	Crop	Commodity	DALA	RP30228		RP32490	
				mg/kg	% of parent	mg/kg	% of parent
		whole plant	0	0.03	0.4%	0.02	0.3%
		roots	20	0.01	9%	<0.01	-
		tops	20	0.04	2%	0.03	1%
		roots	27	0.02	17%	<0.01	-
		tops	27	0.06	3%	0.02	1%
		roots	34	0.01	11%	<0.01	-
		tops	34	0.04	3%	0.02	1%
CA 6.3.1/5	carrot	whole plant	0	0.05	0.4%	<0.01	-
		roots	21	<0.01	-	<0.01	-
		tops	21	0.05	5%	<0.01	-
		roots	28	<0.01	-	<0.01	-
		tops	28	0.06	6%	<0.01	-
		roots	35	<0.01	-	<0.01	-
		tops	35	0.06	9%	<0.01	-
		whole plant	0	0.04	0.4%	0.01	0.1%
		roots	21	<0.01	-	<0.01	-
		tops	21	0.09	3%	0.05	2%
		roots	26	<0.01	-	<0.01	-
		tops	26	0.12	5%	0.03	1%
		roots	34	<0.01	-	<0.01	-
		tops	34	0.06	3%	0.02	1%
		whole plant	0	0.04	1%	<0.01	-
		roots	22	<0.01	-	<0.01	-
		tops	22	0.09	4%	0.02	1%
		roots	29	<0.01	-	<0.01	-
		tops	29	0.09	5%	0.01	1%
		roots	35	<0.01	-	<0.01	-
		tops	35	0.06	4%	0.01	1%
		whole plant	0	0.04	1%	<0.01	-
		roots	20	<0.01	-	<0.01	-
		tops	20	1.01	5%	0.07	0.3%
		roots	27	<0.01	-	<0.01	-
		tops	27	1.09	7%	0.06	0.4%
		roots	35	<0.01	-	<0.01	-
		tops	35	3.83	56%	0.08	1%
		whole plant	0	0.06	1%	<0.01	-
		roots	21	0.02	13%	<0.01	-
		tops	21	0.10	4%	0.02	1%
		roots	27	0.02	14%	<0.01	-
		tops	27	0.08	7%	0.01	1%
		roots	35	0.02	15%	<0.01	-
		tops	35	0.07	5%	0.02	1%
		whole plant	0	0.04	0.5%	<0.01	-
		roots	21	0.01	11%	<0.01	-
		tops	21	0.19	6%	0.05	1%
		roots	26	0.01	12%	<0.01	-
		tops	26	0.12	6%	0.03	2%
		roots	35	0.01	12%	<0.01	-
		tops	35	0.15	6%	0.05	2%
		whole plant	0	0.07	1%	0.03	0.4%
		roots	21	0.05	10%	<0.01	-
		tops	21	0.31	1%	0.50	2%
		roots	29	0.05	11%	0.01	-

Report No	Crop	Commodity	DALA	RP30228		RP32490	
				mg/kg	% of parent	mg/kg	% of parent
		tops	29	0.31	1%	0.33	2%
		roots	35	0.09	19%	0.01	-
		tops	35	0.85	7%	0.28	2%
		whole plant	0	0.08	0.2%	0.01	0.04%
		roots	20	0.02	8%	0.01	-
		tops	20	0.08	3%	0.03	1%
		roots	27	0.01	9%	0.01	-
		tops	27	0.08	4%	0.02	1%
		roots	34	0.01	12%	0.01	-
		tops	34	0.06	3%	0.01	-
CA 6.3.2/5	lettuce	wh. plant	0	0.03	0.2%	0.03	0.2%
		head	14	0.02	6%	0.03	9%
		head	21	<0.01	-	<0.01	-
		head	28	<0.01	-	<0.01	-
		wh. plant	0	0.05	0.4%	0.05	0.4%
		head	13	0.12	10%	0.08	7%
		head	21	<0.01	-	0.01	25%
		head	28	<0.01	-	<0.01	-
		wh. plant	0	0.03	0.3%	<0.01	-
		head	14	<0.01	-	<0.01	-
		head	21	<0.01	-	<0.01	-
		head	28	<0.01	-	<0.01	-
		wh. plant	0	0.04	0.2%	0.05	0.2%
		head	14	<0.01	-	0.08	3%
		head	21	0.01	2%	0.04	8%
		head	27	<0.01	-	0.01	14%
		wh. plant	0	0.09	0.4%	0.11	0.4%
		head	14	0.06	9%	0.09	14%
		head	21	0.03	13%	0.06	25%
		head	28	0.01	17%	0.02	33%
		wh. plant	0	0.03	0.2%	0.02	0.2%
		head	14	0.02	3%	0.05	9%
		head	21	0.02	9%	0.05	22%
		head	27	<0.01	-	0.01	33%
		wh. plant	0	0.02	0.1%	0.01	0.1%
		head	13	0.01	2%	0.03	5%
		head	21	<0.01	-	<0.01	-
		head	28	<0.01	-	<0.01	-
		wh. plant	0	<0.01	-	<0.01	-
		head	14	<0.01	-	<0.01	-
		head	21	<0.01	-	<0.01	-
		head	28	<0.01	-	<0.01	-

Report No	Crop	Commodity	DALA	RP30228		RP32490	
				mg/kg	% of parent	mg/kg	% of parent
CA 6.3.2/6	lettuce	head	0	0.12	1%	0.05	0.4%
		head	14	0.02	10%	0.03	11%
		head	21	<0.01	-	0.01	24%
		head	27	<0.01	-	<0.01	-
		head	0	0.04	0.2%	0.02	0.1%
		head	13	0.02	5%	0.03	7%
		head	20	<0.01	-	0.01	12%
		head	27	<0.01	-	<0.01	-
		head	0	0.03	0.2%	0.03	0.2%
		head	13	0.04	6%	0.05	8%
		head	21	0.01	18%	0.01	23%
		head	28	<0.01	-	<0.01	-
		head	0	0.06	0.3%	0.05	0.2%
		head	13	0.13	4%	0.06	2%
		head	21	0.08	5%	0.07	5%
		head	28	0.06	5%	0.06	5%
		head	0	0.08	0.3%	0.08	0.3%
		head	14	0.02	2%	0.04	4%
		head	21	<0.01	-	0.02	7%
		head	28	<0.01	-	0.01	15%
		head	0	0.08	0.4%	0.04	0.2%
		head	14	0.02	24%	0.02	28%
		head	21	<0.01	-	0.01	31%
		head	28	<0.01	-	0.05	-
		head	0	0.02	0.1%	0.01	0.1%
		head	15	0.05	26%	0.03	16%
		head	21	<0.01	-	0.01	-
		head	27	<0.01	-	0.01	-
		head	0	0.06	0.3%	0.03	0.1%
		head	14	0.02	13%	0.03	18%
		head	21	<0.01	-	0.01	-
		head	28	<0.01	-	0.01	-
CA 6.3.2/7	lettuce	leaves	0	0.14	1%	0.04	0.2%
		leaves	7	0.10	1%	0.04	0.2%
		leaves	14	0.08	1%	0.05	0.3%
		leaves	21	0.02	1%	0.02	0.4%
		leaves	0	0.10	1%	0.02	0.1%
		leaves	7	0.57	6%	0.06	1%
		leaves	14	0.28	7%	0.05	1%
		leaves	21	0.09	16%	0.03	4%
		leaves	0	0.33	2%	0.04	0.2%
		leaves	7	0.16	1%	0.04	0.3%
		leaves	14	0.16	2%	0.03	0.4%
		leaves	21	0.13	2%	0.03	0.4%
		leaves	0	0.14	1%	0.04	0.2%
		leaves	7	0.13	9%	0.09	6%
		leaves	14	0.07	13%	0.06	12%
		leaves	21	0.05	116%	0.02	35%
		leaves	0	0.00	1%	<0.01	-
		leaves	7	0.11	0.5%	0.06	0.2%
		leaves	14	0.14	1%	0.07	1%
		leaves	21	0.10	2%	0.05	1%

Report No	Crop	Commodity	DALA	RP30228		RP32490	
				mg/kg	% of parent	mg/kg	% of parent
		leaves	0	0.18	1%	0.04	0.1%
		leaves	7	0.11	1%	0.04	0.2%
		leaves	14	0.07	0.5%	0.05	0.3%
		leaves	21	0.06	1%	0.05	0.5%
		leaves	0	0.52	1%	0.03	0.1%
		leaves	7	0.96	5%	0.05	0.3%
		leaves	14	0.58	5%	0.04	0.4%
		leaves	21	0.52	6%	0.05	1%
		leaves	0	2.00	10%	0.03	0.1%
		leaves	7	2.00	23%	0.03	0.3%
		leaves	14	1.00	22%	0.03	1%
		leaves	21	0.26	30%	0.01	1%
2010/1007192	broccoli	leaves	0	<0.01	-	<0.01	-
		leaves	7	0.01	5%	<0.01	-
		leaves	13	<0.01	-	<0.01	-
		leaves	20	<0.01	-	<0.01	-
		leaves	0	<0.01	-	<0.01	-
		leaves	7	<0.01	-	<0.01	-
		leaves	14	<0.01	-	<0.01	-
		leaves	21	<0.01	-	<0.01	-
		leaves	0	0.01	0.3%	<0.01	-
2011/1120987	broccoli	leaves	8	<0.01	-	<0.01	-
		leaves	15	<0.01	-	<0.01	-
		leaves	22	<0.01	-	<0.01	-
		leaves	0	<0.01	-	0.02	0.2%
		leaves	8	<0.01	-	<0.01	-
		leaves	15	<0.01	-	<0.01	-
		leaves	20	<0.01	-	<0.01	-
2010/1007191	tomato	fruit	0	<0.01	-	0.01	1%
		fruit	1	<0.01	-	0.02	2%
		fruit	3	<0.01	-	0.01	6%
		fruit	7	<0.01	-	0.01	4%
		fruit	0	0.01	1%	0.02	2%
		fruit	1	0.01	1%	0.02	2%
		fruit	4	0.01	1%	0.03	3%
		fruit	8	<0.01	-	0.02	14%
		fruit	0	<0.01	-	0.03	2%
		fruit	1	<0.01	-	0.01	1%
		fruit	3	<0.01	-	0.01	1%
		fruit	7	<0.01	-	0.01	1%
		fruit	0	<0.01	-	0.04	23%
		fruit	1	<0.01	-	0.03	26%
		fruit	3	<0.01	-	0.04	21%
		fruit	7	<0.01	-	0.04	28%

Report No	Crop	Commodity	DALA	RP30228		RP32490	
				mg/kg	% of parent	mg/kg	% of parent
2011/1120983	tomato	fruit	0	<0.01	-	0.04	8%
		fruit	1	<0.01	-	0.04	5%
		fruit	3	<0.01	-	0.03	7%
		fruit	7	<0.01	-	0.01	20%
		fruit	0	<0.01	-	0.03	2%
		fruit	1	<0.01	-	0.04	2%
		fruit	3	<0.01	-	0.04	3%
		fruit	7	<0.01	-	0.05	3%
		fruit	0	<0.01	-	0.03	2%
		fruit	1	<0.01	-	0.03	2%
		fruit	3	<0.01	-	0.04	2%
		fruit	7	<0.01	-	0.03	2%
		fruit	0	<0.01	-	0.03	8%
		fruit	1	<0.01	-	0.03	17%
		fruit	3	<0.01	-	0.04	24%
		fruit	6	<0.01	-	0.04	20%
2010/1007193	plum	fruit	0	<0.01	-	<0.01	-
		fruit	8	<0.01	-	<0.01	-
		fruit	15	<0.01	-	<0.01	-
		fruit	21	<0.01	-	<0.01	-
		fruit	0	<0.01	-	<0.01	-
		fruit	6	<0.01	-	0.01	5%
		fruit	13	<0.01	-	0.02	7%
		fruit	20	<0.01	-	<0.01	-
		fruit	0	<0.01	-	<0.01	-
		fruit	6	<0.01	-	0.01	5%
		fruit	13	<0.01	-	<0.01	-
		fruit	20	<0.01	-	0.01	9%
		fruit	0	<0.01	-	<0.01	-
		fruit	6	<0.01	-	<0.01	-
		fruit	13	<0.01	-	<0.01	-
		fruit	20	<0.01	-	0.01	2%
		fruit	0	<0.01	-	<0.01	-
		fruit	7	<0.01	-	<0.01	-
		fruit	14	<0.01	-	<0.01	-
		fruit	21	<0.01	-	<0.01	-
		fruit	0	<0.01	-	<0.01	-
		fruit	7	<0.01	-	<0.01	-
		fruit	14	<0.01	-	<0.01	-
		fruit	20	<0.01	-	<0.01	-
		fruit	0	<0.01	-	<0.01	-
		fruit	7	<0.01	-	<0.01	-
		fruit	14	<0.01	-	<0.01	-
		fruit	20	<0.01	-	<0.01	-

Report No	Crop	Commodity	DALA	RP30228		RP32490	
				mg/kg	% of parent	mg/kg	% of parent
2011/1120982	plum	fruit	0	<0.01	-	<0.01	-
		fruit	6	<0.01	-	<0.01	-
		fruit	13	<0.01	-	<0.01	-
		fruit	20	<0.01	-	<0.01	-
		fruit	0	<0.01	-	<0.01	-
		fruit	7	<0.01	-	<0.01	-
		fruit	14	<0.01	-	<0.01	-
		fruit	21	<0.01	-	<0.01	-
		fruit	0	<0.01	-	<0.01	-
		fruit	8	<0.01	-	<0.01	-
		fruit	15	<0.01	-	<0.01	-
		fruit	22	<0.01	-	<0.01	-
		fruit	0	<0.01	-	<0.01	-
		fruit	7	<0.01	-	<0.01	-
		fruit	14	<0.01	-	0.01	3%
		fruit	21	<0.01	-	0.02	8%
		fruit	0	<0.01	-	<0.01	-
		fruit	7	<0.01	-	0.01	2%
		fruit	15	0.01	4%	<0.01	-
		fruit	21	0.02	3%	<0.01	-
		fruit	0	<0.01	-	<0.01	-
		fruit	6	<0.01	-	<0.01	-
		fruit	14	<0.01	-	<0.01	-
		fruit	20	<0.01	-	<0.01	-
		fruit	0	<0.01	-	<0.01	-
		fruit	7	<0.01	-	<0.01	-
		fruit	15	<0.01	-	<0.01	-
		fruit	21	<0.01	-	<0.01	-
		Overall mean			**6%**		**5%**
		Mean (without day< 0 results)			**8%**		**6%**

CA 6.4 Feeding studies

Intended uses with the representative formulation BAS 610 06 F covered in this submission include carrot and lettuce which are both are not relevant feed items.
As a chronic consumer risk assessment based on only these crops does not allow a realistic overall assessment a second scenario was calculated comprising all uses intended to be defended in future.
Feeding studies necessary as basis for these calculations were all peer-reviewed earlier. No new studies were conducted.

CA 6.4.1 Poultry

During the peer review under Directive 91/414/EEC, the magnitude of iprodione residues in poultry was investigated in a feeding study with laying hens (C021535).

In this study, iprodione plus its non-hydroxylated metabolites in poultry were determined with laying hens (France, 1996). Four groups of laying hens consisting of ten animals were dosed for 28 days with iprodione at levels of 2, 20 and 100 mg/kg in the diet (equivalent to 0.15, 1.5 and 7.5 mg/kg bw per d). Samples were analysed for iprodione and its non-hydroxylated metabolites convertible into 3,5-dichloroaniline. Hydroxylated metabolites were not identified in the poultry metabolism study therefore were not analysed for in the poultry feeding study.

CA 6.4.2 Ruminants

During the peer review under Directive 91/414/EEC, the magnitude of iprodione residues in ruminants was investigated in a feeding study with lactating cows (C021585).

In this study, four groups of lactating cows, each consisting of three animals were dosed for 29 consecutive days with iprodione at levels of 5, 15, 50 and 200 mg/kg in the diet (equivalent to 0.180, 0.540, 1.81 and 7.27 mg/kg bw). Milk samples were analyzed for iprodione, its non-hydroxylated metabolites and its hydroxylated metabolites 8, 17 and 28 days after treatment. Total residues (sum of iprodione, its non-hydroxylated metabolites convertible into 3,5-dichloroaniline and its hydroxylated metabolites convertible into 3,5-dichloro-4-hydroxy-aniline) were expressed as iprodione equivalents. Residues in milk were demonstrated to be above the LOQ for the sum of hydroxylated and non-hydroxylated metabolites.

CA 6.4.3 Pigs

No feeding studies in pigs were performed.

CA 6.4.4 Fish

No feeding studies in fish were performed.

Please also see explanation in CA 6.2.5, metabolism in fish.

CA 6.5 Effects of Processing

The effect of processing on the nature of iprodione was investigated during the initial EU Review. Studies were conducted using four test conditions (30 minutes at 70°C, pH 4 and 6; 30 minutes at 130°C, pH 4 and 6). Whilst iprodione was found to be stable at 70°C (pH 4 and 6) and at 130°C (pH 4), iprodione degraded almost completely at 130°C (pH 6), conditions representative of sterilisation, with the notable formation of 3,5-dichloroaniline and a range of other metabolites.
As the conditions used in the former study do not fully comply with the present guideline, a new study fulfilling OECD Guideline 507 was conducted.

CA 6.5.1 Nature of the residue

Report: CA 6.5.1/1
Adam D., 2011a
^{14}C-Iprodione: Simulated processing - Aqueous hydrolysis at 90, 100 and 120°C
2011/1285129
Guidelines: OECD Test Guideline 507 - Nature of the residues in processed commodities - High temperature hydrolysis, EPA 860.1520, EEC 7035/VI/95 rev. 5
GLP: Yes
(certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Executive Summary

The results show that during processing in pH 4, 90 °C, pH 5, 100 °C and pH 6 120 °C simulating pasteurisation, baking/brewing/boiling and sterilisation, respectively, Iprodione is not expected to degrade hydrolytically in pH 4. With increasing pH and at a higher temperature the formation of RP30228 is favoured followed by ring opening to RP37176 and further degradation to RP32596.

I. MATERIAL AND METHODS

1. Test item

Internal code:	BAS 610 F
Reg.No.:	101169
Chem. name:	3-(3,5-dichlorophenyl)-N-isopropyl-2,4-dioxo-imidazolidine-1-carboxamide
Molar mass:	330.2 g/mol (unlabelled)
Label:	^{14}C-Iprodione (phenyl-U-C^{14})
Batch-No.:	967-1001
Specific act.:	6.69 MBq/mg
Radiochem. Purity:	95.3%

2. Test system

The study was performed in buffer solutions at three different pH-values chosen to simulate normal processing practice.

pH 4 acetate buffer: 370 mL 0.1 M acetic acid was adjusted to pH 4 with 125 mL 0.1 M sodium acetate and diluted up to 1L with sterilised water.
pH 5 acetate buffer: 50 mL 0.1 M acetic acid was adjusted to pH 5 with 125 ml 0.1 M M sodium acetate and diluted up to 75 ml with sterilised water .
pH 6 acetate buffer: 10 mL 0.1 M acetic acid was adjusted to pH 6 with 250 mL 0.1 M sodium acetate and diluted up to 1L with sterilised water.

The final concentrations of the buffer solutions were about 0.05 mol/L. All buffer solutions were sterilised by filtration.

3. Description of analytical procedures

The quantity of radioactivity was determined by Packard liquid scintillation counters equipped with DPM and luminescence options. HPLC was the primary analytical method used to determine the amount of test item and any degradation products in the samples. TLC was the secondary analytical method used to determine the amount of test item and degradation products in the samples.

4. Study Design

The experiment was conducted using duplicate samples of 10 mL sterile buffer solution containing 14C-Iprodione. The samples were incubated under the following conditions.

pH 4: At 90°C for 20 minutes in closed pear shaped flasks using an oil bath, representing the process of pasteurisation.
pH 5: At 100°C for 60 minutes in closed pear shaped flasks using an oil bath, representing the processes of baking, brewing and boiling.
pH 6: At 120°C for 20 minutes in closed pear shaped flasks using an oil bath, representing the process of sterilisation.

The temperature of the solutions was continuously monitored by a thermo element placed in the oil bath.

After incubation, the pH value of the samples was measured and directly submitted for LSC measurements, HPLC and TLC analysis, to determine the amount of 14C-Iprodione and hydrolysis products in the corresponding buffer solutions. The weight of each sample was determined immediately after sampling.

II. RESULTS AND DISCUSSION

The overall radioactivity before and after each test performance is given in Table 6.5.1-1. No major loss of radioactive material occurred. The amount of radioactive material in the samples at the end of each test corresponded to iprodione only. No degradation products were detected by radio-HPLC analysis.

Table 6.5.1-1: Material balance before and after processing simulation tests with ^{14}C-BAS Iprodione

Test	pH 4, 90°C	pH 5, 100°C	pH 6, 120°C
before test [%TAR]	100.0	100.0	100.0
after test [% TAR]	100.4	102.1	103.7

TAR = total applied radioactivity

^{14}C-Iprodione and the radioactive fractions were identified by co-elution with unlabelled test and reference items using HPLC and TLC. The quantitative determination was carried out based on the results of the HPLC analysis. The results obtained by HPLC were confirmed by TLC.

The results are presented in Table 6.5.1-2 in percent of the applied radioactivity.

pH 4
Iprodione was shown to be rather stable to hydrolysis at pH 4 and 90°C, representing a mean amount of 94.5% after 20 minutes of incubation. Metabolite RP30228 amounted to 3.0%, RP32596 to 1.3% and metabolite RP37176 accounted for 1.6% of the applied radioactivity.

pH 5
More than 30% of the test item degraded within 60 minutes in pH 5 at 100 °C, with the test item representing mean amounts of 66.6% TAR. RP30228 reached amounts of 30.3%. RP32596 and RP37176 did not exceed 3.3%.

pH 6
In pH 6 at 120 °C, the test item degraded rapidly when compared to pH 4 and 5. The test item hydrolysed to 53.0% of TAR after 20 min of incubation. Besides the test item, two major and one minor degradates were observed. RP30228 reached mean amounts of 24.8% and RP37176 20.5% of TAR. RP32596 represented 5.5% of TAR.

Table 6.5.1-2: Distribution pattern after hydrolysis of 14C- Iprodione under different conditions

Conditions			Iprodione	RP 30228	RP 37176	RP 32596 (3,5-DCA)
pH	Temp. [°C]	Time [min]	% of total applied radioactivity (TAR)			
4	90	20	94.5	3.0	1.6	1.3
5	100	60	66.6	30.3	3.3	1.2
6	120	20	53.0	24.8	20.5	5.5

III. CONCLUSION

Iprodione is hydrolytically stable in sterile buffer solution pH 4 at a temperature simulating pasteurisation (90 °C).
The test item degraded under the conditions in pH 5 at 100 °C after 60 minutes (simulating baking/brewing/boiling) and in pH 6 at 120 °C after 20 minutes of incubation simulating the process of sterilisation. Up to three major metabolites were identified, namely RP30228, RP32596 and RP37176.

CA 6.5.2 Distribution of the residue in inedible peel and pulp

Not relevant for the intended uses in carrot and lettuce.

CA 6.5.3 Magnitude of residues in processed commodities

Report:	CA 6.5.3/1 Plier S., 2012a Determination of residues of BAS 610 F (Iprodione) in carrots and their processed products after four applications of BAS 610 06 F in Germany 2011/1248837
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, BBA IV 3-3, BBA IV 3-4, OECD 508 Magnitude of the Pesticide Residues in Processed Commodities (2008), OECD 509 Crop Field Trial (2009), EEC 7029/VI/95 rev. 5 Appendix B (July 22 1997), EEC 7035/VI/95 rev. 5
GLP:	Yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

During the 2010 growing season, four field trials were conducted in order to investigate the residue behavior of BAS 610 F (iprodione) in carrots and their processed products previously treated with the test item BAS 610 06 F. The field trials were located in representative carrot growing areas in Germany. Each field trial consisted of a treated plot. In two field trials a control plot was also included. The test item BAS 610 06 F, a WG formulation of BAS 610 F was foliar applied four times at an exaggerated target rate of 2.25 kg iprodione/ha for each application. The applications were made 56-58 days, 46-48 days, 37-38 days and 27-28 days before harvest.
Carrot specimens for analysis were sampled on the day of last application and 27-28 days after last application. RAC specimens for analysis were taken from the treated specimens directly before the start of processing. The processing of carrots was conducted with carrot specimens taken at the last sampling. After processing, eleven different fractions of carrot products or intermediates were collected for analysis, namely washed carrots, wash water, peeled carrots, peel, cooked carrots, cooking liquid, juice, wet pomace, canned carrots, vegetable stock and blanching water.
The specimens were analyzed for residues of iprodione and its metabolites RP32490, RP30228, RP32596 and RP37176 using BASF method No L0180/01 which has a limit of quantitation of 0.01 mg/kg for each of the analytes. The results of procedural recovery experiments ranged from 94-98% at fortification levels of 0.01-80 mg/kg.
The samples were also analysed for the recently discovered metabolite M610F007 in a separate study. The analytical details are described in see CA 6.3.1/6, the results thereof are also included in this summary.

The processing study was conducted to determine the potential for concentration of residues of **iprodione** and its metabolites which might be formed during industrial processes.

The mean transfer factors representing the different processing steps were below 1 for iprodione in all processed commodities but peel. Therefore it can be concluded, that iprodione is not being accumulated in these processed fractions. The mean transfer factor of peel (3.61) indicates that the iprodione residues are mainly located in the peel of the carrots roots.

The mean transfer factors representing the different processing steps were below 1 for the metabolite **RP30228** in all processed commodities but peel. Therefore it can be concluded, that the metabolite RP30228 is not being accumulated or formed in these processed fractions. The mean transfer factor of peel (3.67) indicates that the metabolite RP30228 residues are mainly located on peel. The mean transfer factor (3.28 and 5.38) determined for the metabolite RP30228 in cooked carrots and canned carrots can be explained by formation of this metabolite at elevated temperatures which is in accordance with the results of the ^{14}C hydrolytic stability study (CA 6.5.1/1).

The mean transfer factors representing the different processing steps were <1 for the metabolite **RP32596** in washed carrots, wash water, peeled carrots, cooking liquid, wet pomace and blanching water. Therefore it can be concluded, that the metabolite RP32596 is not being accumulated or formed in these processed fractions. The mean transfer factor of peel (7.0) indicates that the metabolite RP32596 residues are mainly located in this processed fraction.

The mean transfer factors (1.50, 1.25, 3 and 10) determined for the metabolite RP32596 in juice, vegetable stock, cooked carrots and canned carrots can be explained by formation of this metabolite at elevated temperatures which is in accordance with the results of the ^{14}C hydrolytic stability study (CA 6.5.1/1).

The residues of **M610F007** in processed fractions originating from a previously reported study were analyzed (for analytical details see CA 6.3.1/6). The mean transfer factors representing the different processing steps were below 1 for M610F007 in washed carrots, wash water, peeled carrots, cooked carrots, cooking liquid, juice, canned carrots, vegetable stock, blanching water and at 1.03 for wet pomace.

Therefore it can be concluded, that M610F007 is not being accumulated in these processed fractions. The mean transfer factor of peel (> 1) indicates that the M610F007 residues are mainly located in the peel of the carrot roots.

Transfer factors of the metabolites **RP32490** and **RP37176** were not calculated, because residues were below the LOQ in the RAC as well as in the processed commodity. However, the finding of RP37176 at the LOQ of 0.01 mg/kg in two samples of canned carrots can be explained by formation of this metabolite at elevated temperatures which is in accordance with the results of the ^{14}C hydrolytic stability study (CA 6.5.1/1).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 610 06 F
Lot/Batch #: 09-160008, BAS 610 F (Reg No. 101169): 75% nominal
CAS#: 36734-19-7

2. Test Commodity:

Crop: Carrot
Type: Root and tuber vegetables
Variety: Nantaise 2
Botanical name: *Daucus carota*
Crop parts(s) or processed commodity: Whole plants with roots, roots, washed carrots, wash water, peeled carrots, peel, cooked carrots, cooking liquid, juice, wet pomace, canned carrots, vegetable stock and blanching water

B. STUDY DESIGN

1. Test procedure

During the 2010 growing season, four field trials were conducted in order to investigate the residue behavior of BAS 610 F (iprodione) in carrots and their processed products previously treated with the test item BAS 610 06 F. The field trials were located in representative carrot growing areas in Germany.
Each field trial consisted of a treated plot. In two field trials a control plot was also included. The test item BAS 610 06 F, a WG formulation of iprodione, was foliar applied four times at an exaggerated target rate of 3 kg product/ha (nominal a.s./ha: 2.25 kg iprodione) for each application. The applications were made 56-58 days, 46-48 days, 37-38 days and 27-28 days before harvest using a spray volume of 500 L/ha.

Carrot RAC samples were harvested on day 0 as well as 27-28 days after the last application. They were separately processed using simulated processing procedures to washed carrots, wash water, peeled carrots, peel, cooked carrots, cooking liquid, juice, wet pomace, canned carrots, vegetable stock and blanching water.
The simulated processes were conducted under the following conditions:

Table 6.5.3-1: Processing conditions

Process	Process Phase	Max. Temp. (° C)	Max. Pressure (bar)	Max. time interval (min)	Medium	Product
Cooking	Heating	100	-	15	salt water	cooked carrots, cooking liquid
Blanching	Heating	96.2	-	2	salt water	blanched carrots, blanching water
Sterilisation	Heating	125	-	7	salt water	canned carrots, vegetable stock
Pasteurisation	Heating under pressure	88.4	2-3	2	-	juice, wet pomace

2. Description of analytical procedures

Iprodione and its metabolites RP32490, RP30228, RP32596 and RP37176 were analysed with BAS method No L0180/01. Residues were extracted with a mixture of acetonitrile and water. An aliquot of the extract was centrifuged and diluted with a mixture of acetonitrile, water and formic acid. The final determination of BAS 610 F and its metabolites was performed by UPLC-MS/MS. The method has a limit of quantitation of 0.01 mg/kg for each analyte.
Control samples were fortified with the analytes at concentration between 0.01 and 80 mg/kg and analyzed simultaneously with the treated samples in order to determine the efficiency of the method. Fortification results are listed in the following table:

Table 6.5.3-2: Procedural recoveries

Matrix	Fort. level (mg/kg)	Compound	n	Mean (%)	SD (+/-)	RSD (%)
Whole plant with roots, roots, tops, washed carrots, wash water, peeled carrots, peel, cooked carrots, cooking liquid, juice, wet pomace, canned carrots, vegetable stock, blanching water	0.01-80	BAS 610 F	34	97.4	9.1	9.3
		RP32490	32	94.1	6.3	6.7
		RP30228	34	96.6	8.3	8.6
		RP32596	34	97.5	8.8	9.0
		RP37176	30	98.3	9.5	9.7

II. RESULTS AND DISCUSSION

Carrot root specimens sampled at 27-28 days after last application contained iprodione between 0.32 and 2.02 mg/kg. After processing, the portions destined for human consumption washed carrots (0.27–1.81 mg/kg), peeled carrots (0.08–0.59 mg/kg), cooked carrots (0.04–0.39 mg/kg), juice (0.09–0.63 mg/kg), canned carrots (<0.01–0.09 mg/kg) and vegetable stock (<0.01–0.01 mg/kg) contained less iprodione than the RAC.
The amount of iprodione in the waste fractions wash water (<0.01–0.10 mg/kg), cooking liquid (<0.01–0.06 mg/kg), wet pomace (0.28–1.81 mg/kg) and blanching water (<0.01–0.02 mg/kg) was lower and of peel (1.61–5.68 mg/kg) was higher than in the unprocessed carrots.
Carrot root specimens sampled at 27-28 days after last application contained residues of the metabolite RP30228 between 0.01 and 0.09 mg/kg. After processing, the portions destined for human consumption washed carrots (0.01–0.10 mg/kg), peeled carrots (<0.01–0.03 mg/kg), juice (0.01–0.06 mg/kg) and vegetable stock (<0.01–0.04 mg/kg) contained less residues, cooked carrots (0.02–0.32 mg/kg) and canned carrots (0.07–0.38 mg/kg) contained more residues of the metabolite RP30228 than the RAC.

The amount of the metabolite RP30228 in the waste fractions wash water (<0.01–0.01 mg/kg), cooking liquid (<0.01–0.05 mg/kg) and blanching water (<0.01–0.05 mg/kg) was lower and of peel (0.05–0.25 mg/kg) and wet pomace (0.01–0.08 mg/kg) was higher than in the unprocessed carrots.

Carrot root specimens sampled at 27-28 days after last application contained residues of the metabolite RP32596 between <0.01 and 0.01 mg/kg. After processing, the portions destined for human consumption washed carrots (<0.01 mg/kg) and peeled carrots (<0.01 mg/kg) contained residues at the same level, cooked carrots (0.02–0.04 mg/kg), juice (0.01–0.02 mg/kg), canned carrots (0.04–0.15 mg/kg) and vegetable stock (<0.01–0.02 mg/kg) contained more residues of the metabolite RP32596 than the RAC.
The amount of the metabolite RP32596 in the waste fractions wash water (<0.01 mg/kg), cooking liquid (<0.01 mg/kg) and blanching water (<0.01 mg/kg) was at the same level and of peel (0.02–0.18 mg/kg) was higher than in the unprocessed carrots. The amount in the waste fraction wet pomace (<0.01-0.01 mg/kg) was equal to the unprocessed carrots.
Carrot root specimens sampled at 27-28 days after last application as well as all processed specimens contained no residues of the metabolites RP32490 and RP37176 above the LOQ (exception: canned carrot specimens of trials L100257 and L100260: 0.01 mg/kg).
No residues above the limit of quantitation (0.01 mg/kg) were found in the untreated specimens.

Table 6.5.3-3: Summary of iprodione residues and transfer factors

Processed commodity	Residues of BAS 610 F**[mg/kg]				Transfer factor BAS 610 F				
Trial*	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**Mean**
Whole plant with roots; 0 DALA	46.4	55.6	27.8	21.4	-	-	-	-	-
Roots, 27-28 DALA[1]	1.55	0.59	0.31	1.23	-	-	-	-	-
Tops, 27-28 DALA	8.24	12.2	8.0	7.34	-	-	-	-	-
Roots, RAC[2]	2.02	1.04	0.23	1.02	1	1	1	1	1
Washed carrots	1.81	0.70	0.27	0.92	0.90	0.67	0.84	0.90	0.83
Wash water	0.10	0.03	<0.01	0.01	0.05	0.03	0.03	0.01	0.03
Peeled carrots	0.59	0.35	0.08	0.36	0.29	0.34	0.25	0.35	0.31
Peel	5.68	3.64	1.61	3.15	2.81	3.50	5.03	3.09	3.61
Cooked carrots	0.39	0.17	0.04	0.28	0.19	0.16	0.13	0.27	0.19
Cooking liquid	0.06	0.02	<0.01	0.03	0.03	0.02	0.03	0.03	0.03
Juice	0.63	0.35	0.09	0.29	0.31	0.34	0.28	0.28	0.30
Wet pomace	1.81	0.94	0.28	0.89	0.90	0.90	0.88	0.87	0.89
Canned carrots	0.09	0.03	<0.01	0.03	0.04	0.03	0.03	0.03	0.03
Vegetable stock	0.01	<0.01	<0.01	<0.01	0.00	0.01	0.03	0.01	0.01
Blanching water	0.02	0.01	<0.01	0.02	0.01	0.01	0.03	0.02	0.02

* Trial 1: L100257, Trial 2: L100258, Trial 3: L100259, Trial 4: L100260
** For calculation purposes, <0.01 mg/kg is set 0.01 mg/kg
1 Specimens were taken on the field
2 Specimens were taken directly before start of processing

The mean transfer factors representing the different processing steps were below 1 for iprodione in washed carrots, wash water, peeled carrots, cooked carrots, cooking liquid, juice, wet pomace, canned carrots, vegetable stock and blanching water. Therefore it can be concluded, that iprodione is not being accumulated in these processed fractions.
The mean transfer factor of peel (>1) indicates that the iprodione residues are mainly located in the peel of the carrots roots.

Table 6.5.3-4: Summary of metabolite RP30228 residues and transfer factors

Processed commodity	Residues of RP30228** [mg/kg]				Transfer factor RP30228				
Trial*	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**Mean**
Whole plant with roots; 0 DALA	0.19	0.16	0.06	0.13	-	-	-	-	-
Roots, 27-28 DALA[1]	0.06	0.03	0.01	0.08	-	-	-	-	-
Tops, 27-28 DALA	0.13	0.27	0.15	0.11	-	-	-	-	-
Roots, RAC[2]	0.08	0.05	0.01	0.09	1	1	1	1	1
Washed carrots	0.10	0.04	0.01	0.07	1.25	0.80	1.00	0.78	0.96
Wash water	0.01	<0.01	<0.01	<0.01	0.13	0.20	1.00	0.11	0.36
Peeled carrots	0.03	0.02	<0.01	0.03	0.38	0.40	1.00	0.33	0.53
Peel	0.25	0.20	0.05	0.23	3.13	4.00	5.00	2.56	3.67
Cooked carrots	0.32	0.24	0.02	0.21	4.00	4.80	2.00	2.33	3.28
Cooking liquid	0.05	0.05	<0.01	0.05	0.63	1.00	1.00	0.56	0.80
Juice	0.06	0.04	0.01	0.04	0.75	0.80	1.00	0.44	0.75
Wet pomace	0.08	0.06	0.01	0.08	1.00	1.20	1.00	0.89	1.02
Canned carrots	0.36	0.29	0.07	0.38	4.50	5.80	7.00	4.22	5.38
Vegetable stock	0.04	<0.01	<0.01	<0.01	0.50	0.20	1.00	0.11	0.45
Blanching water	0.03	0.05	<0.01	0.03	0.38	1.00	1.00	0.33	0.68

* Trial 1: L100257, Trial 2: L100258, Trial 3: L100259, Trial 4: L100260
** For calculation purposes, <0.01 mg/kg is set 0.01 mg/kg
1 Specimens were taken on the field
2 Specimens were taken directly before start of processing

The mean transfer factors representing the different processing steps were below 1 for the metabolite RP30228 in washed carrots, wash water, peeled carrots, cooking liquid, juice, vegetable stock and blanching water. Therefore it can be concluded, that the metabolite RP30228 is not being accumulated or formed in these processed fractions.
The mean transfer factor of peel (>1) indicates that the metabolite RP30228 residues are mainly located on peel.
The mean transfer factor (>1) determined for the metabolite RP30228 in cooked carrots and canned carrots can be explained by formation of this metabolite at elevated temperatures which is in accordance with the results of the ^{14}C-hydrolytic stability study (CA 6.5.1/1).

Table 6.5.3-5: Summary of metabolite RP32596 residues and transfer factors

Processed commodity	Residues of RP32596** [mg/kg]				Transfer factor RP32596				
Trial*	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**Mean**
Whole plant with roots; 0 DALA	<0.01	<0.01	0.01	0.01	-	-	-	-	-
Roots, 27-28 DALA[1]	<0.01	<0.01	<0.01	<0.01	-	-	-	-	-
Tops, 27-28 DALA	<0.01	<0.01	<0.01	<0.01	-	-	-	-	-
Roots, RAC[2]	<0.01	<0.01	<0.01	0.01	1	1	1	1	1
Washed carrots	<0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Wash water	<0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Peeled carrots	<0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Peel	0.02	0.18	0.04	0.04	2	18	4	4	7
Cooked carrots	0.02	0.04	0.03	0.03	2	4	3	3	3
Cooking liquid	<0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Juice	0.01	0.02	0.01	0.02	1	2	1	2	1.5
Wet pomace	<0.01	0.01	<0.01	<0.01	1	1	1	1	1
Canned carrots	0.04	0.15	0.07	0.14	4	15	7	14	10
Vegetable stock	0.01	0.01	<0.01	0.02	1	1	1	2	1.25
Blanching water	<0.01	<0.01	<0.01	<0.01	1	1	1	1	1

* Trial 1: L100257, Trial 2: L100258, Trial 3: L100259, Trial 4: L100260
** For calculation purposes, <0.01 mg/kg is set 0.01 mg/kg
1 Specimens were taken on the field
2 Specimens were taken directly before start of processing

The mean transfer factors representing the different processing steps were <1 for the metabolite RP32596 in washed carrots, wash water, peeled carrots, cooking liquid, wet pomace and blanching water. Therefore it can be concluded, that the metabolite RP32596 is not being accumulated or formed in these processed fractions.
The mean transfer factor (>1) determined for the metabolite RP32596 in juice, vegetable stock, cooked carrots and canned carrots can be explained by formation of this metabolite at elevated temperatures which is in accordance with the results of the ^{14}C hydrolytic stability study (CA 6.5.1/1)

Table 6.5.3-6: Summary of metabolite M610F007 residues and transfer factors

Processed commodity	Residues of M610F007** [mg/kg]				Transfer factor M610F007				
Trial*	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**Mean**
Roots, RAC[2]	0.47	0.71	0.56	1.12	-	-		-	-
Washed carrots	0.59	0.72	0.32	0.83	1.26	1.01	0.57	0.74	0.90
Wash water	<0.010	<0.010	<0.010	<0.010	-	-	-	-	-
Peeled carrots	0.18	0.48	0.61	0.37	0.38	0.68	1.09	0.33	0.62
Peel	0.74	1.6	0.97	2.9	1.57	2.25	1.73	2.59	2.04
Cooked carrots	0.13	0.32	0.29	0.37	0.28	0.45	0.52	0.33	0.39
Cooking liquid	0.096	0.24	0.064	0.24	0.20	0.34	0.11	0.21	0.22
Juice	0.32	0.67	0.27	0.81	0.68	0.94	0.48	0.72	0.71
Wet pomace	0.49	0.98	0.44	1.0	1.04	1.38	0.79	0.89	1.03
Canned carrots	0.025	0.059	0.077	0.028	0.053	0.083	0.14	0.025	0.075
Vegetable stock	0.021	0.034	0.045	0.018	0.045	0.048	0.080	0.016	0.047
Blanching water	0.024	0.091	0.028	0.098	0.05	0.13	0.050	0.088	0.079

* Trial 1: L100257, Trial 2: L100258, Trial 3: L100259, Trial 4: L100260
** For calculation purposes, <0.01 mg/kg is set 0.01 mg/kg
1 Specimens were taken on the field
2 Specimens were taken directly before start of processing

The residues of M610F007 in the processed fractions of this study were analyzed in a separate study (for analytical details see CA 6.3.1/6), but the results are included in this summary.

The mean transfer factors representing the different processing steps were below 1 for M610F007 in washed carrots, wash water, peeled carrots, cooked carrots, cooking liquid, juice, canned carrots, vegetable stock, blanching water and at 1.03 for wet pomace.

Therefore it can be concluded, that M610F007 is not being accumulated in these processed fractions.

The mean transfer factor of peel (> 1) indicates that the M610F007 residues are mainly located in the peel of the carrot roots. Considering that carrot roots are not directly hit by the spray solution, this finding could indicate that M610F007 or its precursor 3,5-dichloroaniline were taken up from the soil.

Table 6.5.3-7: Summary of metabolite RP32490 and RP37176 residues

Processed commodity	Residues of RP32490** [mg/kg]				Residues of RP37176** [mg/kg]			
Trial*	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Whole plant with roots; 0 DALA	0.15	0.07	0.03	0.03	0.01	<0.01	<0.01	<0.01
Roots, 27-28 DALA[1]	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tops, 27-28 DALA	0.13	0.09	0.04	0.05	<0.01	<0.01	<0.01	<0.01
Roots, RAC[2]	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01
Washed carrots	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Wash water	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Peeled carrots	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Peel	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cooked carrots	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cooking liquid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Juice	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Wet pomace	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Canned carrots	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	0.01
Vegetable stock	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Blanching water	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

* Trial 1: L100257, Trial 2: L100258, Trial 3: L100259, Trial 4: L100260
** For calculation purposes, <0.01 mg/kg is set 0.01 mg/kg
1 Specimens were taken on the field
2 Specimens were taken directly before start of processing

Transfer factors of the metabolites RP32490 and RP37176 were not calculated, because residues were below the LOQ in the RAC as well as in the processed commodity.
However, the finding of RP37176 at the LOQ of 0.01 mg/kg in two samples of canned carrots can be explained by formation of this metabolite at elevated temperatures which is in accordance with the results of the ^{14}C hydrolytic stability study (CA 6.5.1/1).

III. CONCLUSION

The processing study was conducted to determine the potential for concentration of residues of **iprodione** and its metabolites which might be formed during industrial processes.
The mean transfer factors representing the different processing steps were below 1 for iprodione in all processed commodities but peel. Therefore it can be concluded, that iprodione is not being accumulated in these processed fractions. The mean transfer factor of peel (3.61) indicates that the iprodione residues are mainly located in the peel of the carrots roots.
The mean transfer factors representing the different processing steps were below 1 for the metabolite **RP30228** in all processed commodities but peel. Therefore it can be concluded, that the metabolite RP30228 is not being accumulated or formed in these processed fractions. The mean transfer factor of peel (3.67) indicates that the metabolite RP30228 residues are mainly located on peel. The mean transfer factor (3.28 and 5.38) determined for the metabolite RP30228 in cooked carrots and canned carrots can be explained by formation of this metabolite at elevated temperatures which is in accordance with the results of the ^{14}C hydrolytic stability study (CA 6.5.1/1).
The mean transfer factors representing the different processing steps were <1 for the metabolite **RP32596** in washed carrots, wash water, peeled carrots, cooking liquid, wet pomace and blanching water. Therefore it can be concluded, that the metabolite RP32596 is not being accumulated or formed in these processed fractions. The mean transfer factor of peel (7.0) indicates that the metabolite RP32596 residues are mainly located in this processed fraction.
The mean transfer factors (1.50, 1.25, 3 and 10) determined for the metabolite RP32596 in juice, vegetable stock, cooked carrots and canned carrots can be explained by formation of this metabolite at elevated temperatures which is in accordance with the results of the ^{14}C hydrolytic stability study (CA 6.5.1/1).
Transfer factors of the metabolites **RP32490** and **RP37176** were not calculated, because residues were below the LOQ in the RAC as well as in the processed commodity. However, the finding of RP37176 at the LOQ of 0.01 mg/kg in two samples of canned carrots can be explained by formation of this metabolite at elevated temperatures which is in accordance with the results of the ^{14}C hydrolytic stability study (CA 6.5.1/1).

Report: CA 6.5.3/2
Bonnechère A. et al, 2011a
Publication: Effect of household and industrial processing on levels of five pesticide residues and two degradation products in spinach
2012/1366222
Testing date: 2011
Guidelines: none
GLP: no

Information on the behaviour of iprodione under household and industrial processing is also provided in this public literature. It also contains investigations of other plant protection products which are not included in this summary.
This entry was not included in the application.

Executive Summary

During the 2010 growing season, two field trials were conducted in order to investigate the residue behavior of iprodione and 3,5-dichloroaniline (3,5-DCA) in spinach and its processed products previously treated with the test item Rovral 500 SC (BAS 610 10 F). The field trials were located in Belgium. At each trial site, the test item Rovral 500 SC, an SC formulation of iprodione, was spray applied four times at a target rate of 1 kg a.s./ha for each application. The applications were made starting 28 days before harvest with a retreatment interval of 7-8 days (BBCH 12, 14, 43 and 47). Spinach RAC samples were harvested 6 days after the last application. They were separately processed using simulated household and industrial processing procedures into washed, blanched, microwave-cooked and in-pack sterilized leaves.
The specimens were analyzed for residues of iprodione and its metabolite 3,5-dichloroaniline with GC-MS/MS; the LOQ was 0.02 mg/kg for iprodione and 0.01 mg/kg for 3,5-DCA.
The mean transfer factors representing the different processing steps were below 1 for **iprodione** in all processed commodities. Therefore it can be concluded, that iprodione is not being accumulated in these processed fractions.
The mean transfer factor was slightly below 1 for the metabolite **3,5-DCA** in washed leaves. Therefore it can be concluded, that the metabolite 3,5-DCA is not being accumulated or formed in this processed fraction. The mean transfer factors (1.58, 1.94 and 4.44) determined in blanched, cooked and sterilized leaves can be explained by formation of this metabolite at elevated temperatures which is in accordance with the results of the ^{14}C hydrolytic stability study (CA 6.5.1/1).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: Rovral 500 SC (BAS 610 10 F)
Lot/Batch #: Not reported
CAS#: 36734-19-7 (iprodione)

2. Test Commodity:

Crop: Spinach
Type: Leafy vegetables
Variety: Cezanne, SP-916
Botanical name: *Spinacia oleracea*
Crop parts(s) or processed commodity: Leaves, washed leaves, blanched leaves, microwave-cooked leaves and in-pack sterilized leaves

B. STUDY DESIGN

1. Test procedure

During the 2010 growing season, two field trials were conducted in order to investigate the residue behavior of iprodione and 3,5-dichloroaniline (3,5-DCA) in spinach and its processed products previously treated with the test item Rovral 500 SC (BAS 610 10 F). The field trials were located in Belgium.
At each trial site, the test item Rovral 500 SC, an SC formulation of iprodione, was spray applied four times at a target rate of 1 kg a.s./ha for each application. The applications were made starting 28 days before harvest with a retreatment interval of 7-8 days (BBCH 12, 14, 43 and 47). Spinach RAC samples were harvested 6 days after the last application. They were separately processed using simulated household and industrial processing procedures into washed leaves (15°C water for 3 min), blanched leaves (88°C water for 5 min, 15°C water for cooling), microwave-cooked leaves (800 W for 15 min) and in-pack sterilized leaves (40°C in water for 10 min, then 121°C within 8.5-9 min for 56 min).

2. Description of analytical procedures

Iprodione and its degradation product 3,5-dichloroaniline were analysed with GC-MS/MS. Homogenized samples were extracted with acetone and acidified with phosphoric acid, followed by liquid-liquid partitioning with isopropylether. The final determination of BAS 610 F and 3,5-DCA was performed by gas chromatography coupled with a MS/MS detector. The method had a limit of quantitation of 0.02 mg/kg for iprodione and 0.01 mg/kg for 3,5-DCA.
Recoveries during the analysis of spinach were 92.9% (±2.3) for iprodione and 60.0% (±18.5) for 3,5-DCA. For each batch of analysis, a control and a spiked sample were run. In the control, no pesticide >LOQ was found. The concentrations found in the spike sample were reported in the control chart to evaluate the trends and a variation of 2S is accepted (SANCO/10684/2009).

II. RESULTS AND DISCUSSION

Spinach leaf specimens sampled at 6 days after the last application contained iprodione residues at 6.5 and 10.2 mg/kg. After processing, the portions destined for human consumption washed leaves (3.7-5.3 mg/kg), blanched leaves (1.6-3.3 mg/kg), cooked leaves (1.3-2.8 mg/kg) and sterilized leaves (0.20–0.25 mg/kg) contained less iprodione than the RAC.
Spinach leaf specimens sampled at 6 days after the last application contained 3,5-DCA residues of 0.046 mg/kg in both trials. After processing, the portions destined for human consumption washed leaves (0.038-0.051 mg/kg) contained about the same amount, while blanched leaves (0.069-0.076 mg/kg), cooked leaves (0.084-0.094 mg/kg) and sterilized leaves (0.191-0.217 mg/kg) contained more 3,5-DCA than the RAC.

Table 6.5.3-8: Summary of iprodione residues and transfer factors

Processed commodity	Residues of iprodione* [mg/kg]		Transfer factor iprodione		
Spinach line	**Cezanne**	**SP-916**	**Cezanne**	**SP-916**	**Mean**
Spinach RAC (leaves)	6.542	10.200	-	-	-
Washed leaves	3.717	5.292	0.57	0.52	0.55
Blanched leaves	1.557	3.343	0.24	0.33	0.29
Microwave-cooked leaves	1.319	2.793	0.20	0.27	0.24
In-pack sterilized leaves	0.198	0.245	0.03	0.02	0.03

* Mean of two batches

The mean transfer factors representing the different processing steps were below 1 for iprodione in all processing products. Therefore it can be concluded, that iprodione is not being accumulated in these processed fractions.

Table 6.5.3-9: Summary of 3,5-DCA residues and transfer factors

Processed commodity	Residues of 3,5-DCA * [mg/kg]		Transfer factor 3,5-DCA		
Spinach line	**Cezanne**	**SP-916**	**Cezanne**	**SP-916**	**Mean**
Spinach RAC (leaves)	0.046	0.046	-	-	-
Washed leaves	0.051	0.038	1.11	0.83	0.97
Blanched leaves	0.076	0.069	1.65	1.50	1.58
Microwave-cooked leaves	0.094	0.084	2.04	1.83	1.94
In-pack sterilized leaves	0.191	0.217	4.15	4.72	4.44

* 3,5-Dichloroaniline; mean of two batches

The mean transfer factor for 3,5-DCA in washed spinach leaves was slightly below 1. Therefore it can be concluded, that 3,5-DCA is not being accumulated or formed in this processed fraction.
The mean transfer factors (>1) determined in blanched, cooked and sterilized leaves can be explained by formation of this metabolite at elevated temperatures which is in accordance with the results of the ^{14}C-hydrolytic stability study (CA 6.5.1/1).

III. CONCLUSION

The mean transfer factors representing the different processing steps were below 1 for **iprodione** in all processed commodities. Therefore it can be concluded, that iprodione is not being accumulated in these processed fractions.
The mean transfer factor was slightly <1 for the metabolite **3,5-DCA** in washed leaves. Therefore it can be concluded, that the metabolite 3,5-DCA is not being accumulated or formed in this processed fraction. The mean transfer factors (1.58, 1.94 and 4.44) determined in blanched, cooked and sterilized leaves can be explained by formation of this metabolite at elevated temperatures which is in accordance with the results of the ^{14}C hydrolytic stability study (CA 6.5.1/1).

Overall Summary: Effects on Processing

The new study investigating the nature of the residues during processing according to the current guideline qualitatively confirms the former results: in both studies iprodione proves to be stable at pH 4 and medium temperature (70 or 90°C). At pH 5 at 100°C parent is still major compound but the constitutional isomer RP30228 is increasingly formed together with traces of ring-opened metabolite RP37176 and final degradation product RP32596. At pH 6 and 120°C the ratio of parent and RP30228 decrease again in favour of RP37176 and RP32596.

The quantitative differences between the former and the new study, especially regarding the compound of concern RP32596, can partly be explained by the milder conditions of the latter.

However, the difference in quantity of degradation product RP32596 (old study 42%, new study under slightly milder conditions 6%) is irrelevant as these results only apply to the medium of pure solvent without any plant matrix. As seen from the carrot metabolism study as well as from literature, it is known that RP32596 has the strong tendency to form conjugates with naturally occurring amino acids and carbohydrates. Therefore, a quantitative extrapolation from a hydrolysis in pure solvent to processing procedures in plant matrices is not possible.

A processing study was conducted in carrot to determine the potential for concentration of residues of **iprodione** and its metabolites which might be formed during industrial processes.

The mean transfer factors representing the different processing steps were below 1 for iprodione and RP30228 in all processed commodities but peel. Therefore it can be concluded, that both compounds are not accumulated in these processed fractions. The mean transfer factors for peel indicate that the iprodione and RP30228 residues are mainly located in the peel of the carrots roots.

Expected Residues of 3,5-Dichloroaniline after Processing

As it is of special interest to quantify the amount of 3,5-dichloroaniline potentially formed during processing of iprodione containing raw agricultural commodities, an additional calculation can be done:

Normally a transfer factor indicating a potential accumulation during processing is calculated by dividing the concentration in the processed fraction by the concentration of the same compound in the raw agricultural commodity:

$$F_{transfer} = \frac{C_{PF}}{C_{RAC}}$$

In the case of RP32596 this calculation can not be used as the compound is not yet quantifiable in the RAC of the carrot study (CA6.5.2/1) and is mainly formed during processing. Therefore, an alternative conversion factor 2 can be defined:

$$F_{transfer\ 2} = \frac{C_{\ RP32596\ in\ PF}}{C_{parent\ in\ RAC}}$$

This factor indicates how much metabolite RP32596 is to be expected after processing of a plant commodity containing a certain concentration of parent iprodione.

The results of these calculations for edible commodities of interest, cooked and canned carrots and juice, are shown in the following table:

Table 6.5.3-10: Transfer factors 2 for metabolite RP32596 residues in carrot commodities

Processed commodity	Residues [mg/kg]				Transfer factor 2				
Trial	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**Mean**
	parent iprodione								
Roots, RAC	2.02	1.04	0.23	1.02					
	RP32596								
Cooked carrots	0.02	0.04	0.03	0.03	0.010	0.038	0.130	0.029	**0.05**
Juice	0.01	0.02	0.01	0.02	0.005	0.019	0.043	0.020	**0.02**
Canned carrots	0.04	0.15	0.07	0.14	0.020	0.144	0.304	0.137	**0.15**

* Trial 1: L100257, Trial 2: L100258, Trial 3: L100259, Trial 4: L100260

Applying this calculation to the spinach study CA6.5.2/2 leads to the following results:

Table 6.5.3-11: Transfer factors 2 for metabolite RP32596 residues in spinach commodities

Processed commodity	Residues [mg/kg]		Transfer factor 2		
Trial	**1**	**2**	**1**	**2**	**Mean**
	parent iprodione				
Roots, RAC	6.542	10.2			
	RP32596				
Cooked spinach	0.199	0.084	0.030	0.008	**0.02**
Sterilized spinach	0.198	0.245	0.030	0.024	**0.03**

* Trial 1: spinach line Cezanne, Trial 2: spinach line SP-916

In order to quantify residues of 3,5-dichloroaniline being formed during processing of iprodione containing commodities a transfer factor 2 was defined describing the ratio the concentration of 3,5-DCA in PF compared to the concentration of iprodione in the RAC. The transfer factors 2 obtained from the carrot and spinach studies are generally in the same order of magnitude with the carrot results being slightly higher. Therefore, it is proposed to use the following factors for exposure calculations of 3,5-dichloroaniline for all crops used for cooking, sterilizing or juice production:

Transfer Factor 2 (to calculate concentration of 3,5-dichloroaniline in PF from concentration of iprodione in RAC):

Cooked commodity 0.05
Juice 0.02
Canned commodity 0.15

Expected Residues of RP37176 after Processing

Also for the exposure calculation for metabolite RP37176 in connection with the TTC concept (see CA 6.9) a "transfer factor 2" is necessary which allows the estimation of the metabolite concentration in the processed commodity on the basis of the parent concentration in the RAC.

Table 6.5.3-12: Transfer factors 2 for metabolite RP37176 residues in carrot commodities

Processed commodity	Residues [mg/kg]				Transfer factor 2				
Trial	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**Mean**
	parent iprodione								
Roots, RAC	2.02	1.04	0.23	1.02					
	RP37176								
Canned carrots	0.01	<0.01	<0.01	0.01	0.005	0.010	0.043	0.010	**0.02**

* Trial 1: L100257, Trial 2: L100258, Trial 3: L100259, Trial 4: L100260
For calculating purposes, "<0.01" was set "0.01"

It is proposed to use the following factor for exposure calculations of RP37176 for all crops used in sterilizing processes:

Transfer Factor 2 (to calculate concentration of RP37176 in PF from concentration of iprodione in RAC):

Canned commodity 0.02

CA 6.6 Residues in Rotational Crops

CA 6.6.1 Metabolism in rotational crops

The metabolism of iprodione in rotational crops of cereals, root and tuber vegetables, pulses and oilseeds, fruit and fruiting vegetables after application of [^{14}C-phenyl]-labelled iprodione has been evaluated (DAR 1996). The characteristics of these studies are summarised in Table 6.6.1-1.

Table 6.6.1-1: Summary of metabolism studies in rotational crops previously available

Crop group	Crop	Label position	Application and sampling details				Year	DocID
			Method, F or G	Rate (kg a.s./ha)	Sowing intervals (months)	Harvest time		
Leafy vegetables	spinach	^{14}C-phenyl	bare soil, G	4.4	1, 4, 12	maturity	1983	C024394
Root and tuber vegetables	sugar beet	^{14}C-phenyl	bare soil, G	5 x 0.8	4	112 DAP	1977	C024295
	turnip	^{14}C-phenyl	cropped soil (peanuts)	3 x 1.1	4, 8, 12	30, 60, 120 (maturity) DAP	1985	C022622
	radish	^{14}C-phenyl	bare soil, G	4.4	1, 4, 12	maturity	1983	C024394
Pulses and oilseeds	peanut, soybean	^{14}C-phenyl	cropped soil (peanuts)	3 x 1.1	4, 8, 12	30, 60, 120 (maturity) DAP	1985	C022622
Cereals	oats	^{14}C-phenyl	bare soil, G	4.4	1, 4, 12	maturity	1983	C024394
	wheat	^{14}C-phenyl	bare soil, G	5 x 0.8	4	276 DAP, 81 DAP	1977	C024295
					12	106 DAP, 233 DAP, 102 DAP		
	maize	^{14}C-phenyl	cropped soil (peanuts)	3 x 1.1	4, 8, 12	30, 60, 120 (maturity) DAP	1985	C022622

F Field/outdoor
G Glasshouse/protected
DAP days after planting

Residues in rotated crops were mostly found to be low, especially in edible commodities. In other plant parts (pods, straw or leaves) higher levels of residues were seen. Highest TRR level found at 1.9 mg/kg was in bean pods harvested after 112 days and grown on iprodione treated soil aged for 4 months.

The main result from the greenhouse studies with radiolabelled material is the investigation of the nature of the residue in follow crops: Like in the metabolism studies of the target crops it is reported that parent iprodione is found to be the predominant residue followed by the two metabolites RP30228 and RP32490. In lower ratios metabolites RP36112, RP36221 and RP25040 were identified together with considerable amounts of bound residues. All these findings are in full accordance with the metabolism after foliar treatment which leads to the conclusion that the same degradation reactions can be assumed: also in rotated plants ring-opening of the heterocyclic ring under formation of the constitutional isomer RP30228 is the first step observed. In parallel, decarboxylation after the first ring opening leads to RP36221. Subsequently, the isopropyl group is cleaved from the first degradation products yielding RP30115, RP30112 and RP32490. During these degradation steps free NH_2-groups of urea and biuret units are formed which can all be conjugated with naturally occurring amino acids and carbohydrates which explain the amount of bound residues.
There is no indication for deviating reactions. Only the ratio of the degradation products observed in follow crops compared to target crops is slightly shifted towards the metabolites.

In order to investigate the magnitude of residues in follow crops a number of field rotational crop studies were conducted in the past. However, the application patterns of some of these studies differ considerably and in multiple parameters from the GAPs intended at present:

There are two studies available applying iprodione 10 times at a rate of 1.1 kg at intervals of 7 days (C043633, C043636). Even at this high application rate no residues above LOQ are found for instance in sugar beets, tomato, cotton, sorghum, soybean, corn, peas, and cucumber after varying replant intervals. In the same studies quantifiable residues were found in crops like radish, mustard greens or peas. An extrapolation to the expected residue levels under the intended use pattern is not possible.
Even more extreme are the application conditions in study C024246 in which follow crops are analysed after up to 8 treatments of iprodione at 2.2 kg/ha and replant intervals between 2 and 234 days.
While the cases where no residues were found in follow crops under extreme conditions do have some significance, an estimation of the residue levels in case of quantifiable residues using the principle of proportionality is impossible.

Therefore, a new field rotational crop study was performed to investigate the magnitude of expected iprodione residues under the conditions of the intended uses. For this study, vegetable crops with short development times and close crop rotations were chosen.

CA 6.6.2 Magnitude of residues in rotational crops

Report:	CA 6.6.2/1 Gabriel E.J., 2013b Residue behaviour of BAS 610 F on rotational crops: Carrot, cauliflower, lettuce (seeded), spinach after application to the soil of BAS 610 06 F under field conditions in Germany, Belgium, Southern France and Spain, 2010/2011 2012/1321579
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 91/414 (1607/IV/97 Rev. 2), EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7, SANCO/825/00 rev. 7 (17 March 2004), EEC 96/46 (16.07.1996), EEC 7524/VI/95 rev. 2 (July 22 1997)
GLP:	Yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

During the growing season of 2010 and 2011, five field trials were conducted in Northern and Southern Europe as a rotational crop study with carrots, lettuce, cauliflower and spinach. Each field trial consisted of two plots, one control plot and one plot treated once to bare soil with 5.4 kg/ha of BAS 610 06 F (WG), corresponding to 4.05 kg/ha of iprodione.
The application took place at 30-38 days before seeding/planting. Plant samples were collected at growth stage BBCH 41 and 49.
The specimens were analyzed for residues of iprodione (BAS 610 F) and its metabolites RP30228 (Reg. No 5079647), RP32490 (Reg. No 5079628) and RP32596 (3,5-dichloroaniline, Reg. No 85831) according to the BASF method No L0180/01, which determines the residues by means of UPLC-MS/MS. The limit of quantitation (LOQ) was 0.01 mg/kg per analyte.
Iprodione and the two metabolites RP32490 (Reg. No 5079628) and RP32596 (3,5-dichloroaniline, Reg. No 85831) were not found at or above the limit of quantitation (LOO) in any plant matrix. Residues of RP30228 (Reg. No 5079647) were detected in carrot top specimens taken at BBCH growth stage 49 in the range between <0.01 and 0.017 mg/kg but were not found at or above the LOQ for carrot root specimens.

After identification of a new metabolite in carrot roots, the carrot samples were also analysed for M610F007 (Reg. No. 5916256) in a separate study (see CA 6.3.1/6).
In the carrot samples of this rotational crop study, no residues of M610F007 were detected.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 610 06 F (WG)
Lot/Batch #: 08-160018, 09-160007, iprodione: 75% nominal
Purity: Not reported
CAS#: 36734-19-7
Development code:
Spiking levels: 0.01-1.0 mg/kg

2. Test Commodity:

Crop: Carrot
Cauliflower
Spinach
Lettuce

Type: Root and tuber vegetables
Flowering brassica
Leafy vegetables

Variety: Carrot: Metro F1, Narbonne F1, Bayon RZ, Nantesa
Cauliflower: Lecanu, Stella F1, Nautilus, Premia
Spinach: Tirza
Lettuce: Iceberg, Ballerina RZ, Marvilla Verano

Botanical name: Carrot: *Daucus carota* L. ssp. *sativus*
Cauliflower: *Brassica oleracea* var. *botrytis*
Spinach: *Spinacia oleracea*
Lettuce: *Lactuca sativa*

Crop parts: Carrot: whole plant with root; root; tops
Cauliflower: whole plant without roots; inflorescences
Lettuce/spinach: leaves, heads

Sample size: 0.5-2 kg (12 units)

B. STUDY DESIGN

1. Test procedure

During the growing season of 2010 and 2011, five field trials with carrot, lettuce, spinach and cauliflower were conducted in Germany, Belgium, Southern France and Spain in order to determine the magnitude of the residues of iprodione in rotational crops after treatment with BAS 610 06 F.

Five field trials were carried out as in the season of 2010 the lettuce of subplot 4 of trial L100392 had not achieved sufficient growth. Therefore the subplot of this trial was repeated in the season of 2011 with spinach in the field trial L100719.

BAS 610 06 F (WG) was spray-applied at a rate of 5.4 kg/ha corresponding to 4.05 kg/ha of iprodione. The application rate of spray solution was 200 L/ha.

The application took place at 30-38 days before seeding/planting. At the sampling occasion 0 DAT, the untreated specimens were collected immediately before the application of the treated plots. At 0 DAT, a minimum of 10 soil cores was taken from each subplot, as well as 3 petri dishes per treated subplot for application verification. At 30-33 DAT at least 10 soil cores were taken per subplot, as well as 73-179 DAT at sampling event BBCH 49. Whole plant samples of carrots were taken at BBCH 41 (93-128 DAT); at BBCH 49 (121-164 DAT) roots and tops were sampled. Lettuce leaves were sampled at BBCH 41 (79-107 DAT); at BBCH 49 (99-114 DAT) heads were taken. Whole plant samples of cauliflower were taken at BBCH 41 (113-151 DAT); at BBCH 49 (134-179 DAT) inflorescences were sampled. From trial L100719 whole plant samples of spinach were taken at BBCH 41 (65 DAT); at BBCH 49 (73 DAT) leaves were sampled.
All specimens were stored frozen at or below -18°C until analysis for a maximum period of 432 days.

2. Description of analytical procedures

BASF method No L0180/01 was used for the analysis of iprodione and its metabolites Reg. No 5079647, Reg. No 5079628 and Reg. No 85831. The limit of quantitation (LOQ) was 0.01 mg/kg per analyte.
Iprodione (BAS 610 F; Reg. No 101169), RP32490 (Reg. No 5079628), RP30228 (Reg. No 5079647) and RP32596 (Reg. No 85831) were extracted with a mixture of acetonitrile and water. An aliquot of the extract was centrifuged and measured directly. The final determination of iprodione, RP32490, RP30228 and RP32596 was performed by UPLC-MS/MS.

Table 6.6.2-1: Summary of recoveries for iprodione (BAS 610 F) and metabolites RP32490, RP30228 and RP32596

Crop	Matrix	Fortification level (mg/kg)	Summary recoveries					
			n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
Carrot	BASF method No L0180/01		**Iprodione (BAS 610 F)**			**RP30228 (Reg. No 5079647)**		
	Whole plant[1]	0.01-0.10	2	102	N/A	2	99.8	N/A
	Tops	0.01-1.0	3	99.3	4.8	3	95.7	1.6
	Roots	0.01-1.0	3	93.2	2.2	3	102	8.8
	BASF method No L0180/01		**RP32490 (Reg. No 5079628)**			**RP32596 (Reg. No 85831)**		
	Whole plant[1]	0.01-0.10	2	94.0	N/A	2	88.9	N/A
	Tops	0.01-1.0	3	91.3	3.7	3	86.6	2.4
	Roots	0.01-1.0[3]	2	92.7	N/A	3	95.3	3.5
Lettuce	BASF method No L0180/01		**Iprodione (BAS 610 F)**			**RP30228 (Reg. No 5079647)**		
	Leaves	0.01-0.10	2	95.3	N/A	2	96.9	N/A
	Head	0.01-0.1	2	97.7	N/A	2	98.2	N/A
	BASF method No L0180/01		**RP32490 (Reg. No 5079628)**			**RP32596 (Reg. No 85831)**		
	Leaves	0.01-0.10	2	92.5	N/A	2	71.8	N/A
	Head	0.01-0.1	2	101	N/A	2	74.6	N/A
Spinach	BASF method No L0180/01		**Iprodione (BAS 610 F)**			**RP30228 (Reg. No 5079647)**		
	Whole plant[2]	0.01-1.0	3	91.5	9.6	3	93.2	9.0
	Leaves	0.01-1.0	7	89.5	12	7	88.7	9.1
	BASF method No L0180/01		**RP32490 (Reg. No 5079628)**			**RP32596 (Reg. No 85831)**		
	Whole plant[2]	0.01-1.0	3	92.1	11	3	96.7	2.9
	Leaves	0.01-1.0	7	89.4	9.9	7	95.3	3.0
Cauliflower	BASF method No L0180/01		**Iprodione (BAS 610 F)**			**RP30228 (Reg. No 5079647)**		
	Whole plant	0.01-0.1	6	101	9.0	6	103	7.2
	Inflorescences	0.01-0.1	6	97.9	1.7	6	97.2	6.6
	BASF method No L0180/01		**RP32490 (Reg. No 5079628)**			**RP32596 (Reg. No 85831)**		
	Whole plant	0.01-0.1	6	105	12	6	91.6	4.7
	Inflorescences	0.01-0.1	6	101	5.1	6	89.1	2.3

N/A Not applicable
1 With roots
2 Without roots
3 Not for Reg. No 5079628

Analytical details concerning determination of M610F007 are described in CA 6.3.1/6.

II. RESULTS AND DISCUSSION

Iprodione and the two metabolites RP32490 (Reg. No 5079628) and RP32596 (Reg. No 85831) were not found at or above the limit of quantitation (LOO) in any plant matrix. Residues of RP30228 (Reg. No 5079647) were detected in carrot top specimens taken at BBCH growth stage 49 in the range between <0.01 and 0.017 mg/kg but were not found at or above the LOQ for carrot root specimens. Also, the new metabolite M610F007 (Reg. No. 5916256) identified in carrot roots could not be detected in carrot samples of this study.
No residues of any of the analytes above the limit of quantitation were found in any of the analyzed untreated plant specimens.

Table 6.6.2-2: Summary of residues in rotational crops

Crop	Portion analyzed	Growth stage (BBCH)	DAT	Residues (mg/kg)				
				BAS 610 F	RP30228 (Reg. No 5079647)	RP32490 (Reg. No 5079628)	RP32596 (Reg. No 85831)	M610F007 (Reg. No 5916256)
30-33 days replant interval*								
Carrot	Whole plant[1]	41	93-128	<0.01	<0.01-0.063	<0.01	<0.01	-
	Tops	49	121-164	<0.01	<0.01-0.017	<0.01	<0.01	-
	Root	49	121-164	<0.01	<0.01	<0.01	<0.01	<0.01
Lettuce	Leaves	41	79-107	<0.01	<0.01	<0.01	<0.01	-
	Head/leaves	49	99-114	<0.01	<0.01	<0.01	<0.01	-
Spinach	Whole plant[2]	41	65	<0.01	<0.01	<0.01	<0.01	-
	Leaves	49	73	<0.01	<0.01	<0.01	<0.01	-
Cauliflower	Whole plant[2]	41	113-151	<0.01	<0.01	<0.01	<0.01	-
	Inflorescences	49	134-179	<0.01	<0.01	<0.01	<0.01	-

* Exception: Trial L 100392, where the replanting interval was 38 days.
1 With roots
2 Without roots

III. CONCLUSION

The results of the study show that no residues above the limit of quantitation of iprodione or any of its metabolites are taken up into edible parts of follow crops like carrots, lettuce, cauliflower and spinach planted/seeded 30 to 38 days after application of BAS 610 06 F to bare soil.

Table 6.6.2-3: Residues in succeeding crops

Study details		Formulation, appl. rate (kg a.s./ha)	DAA	Crop	Residues (mg/kg)					
					Matrix	BAS 610 F	I	II	III	IV
Study code:	370493				**31/38 day plant back interval**					
DocID:	2012/1321579	BAS 610 06 F	128	Carrot	Whole plant[1]	<0.01	<0.01	<0.01	<0.01	-
GLP:	Yes	1 x 4.05	164		Roots	<0.01	<0.01	<0.01	<0.01	<0.01
Year:	2010	to bare soil	164		Tops	<0.01	<0.01	<0.01	<0.01	-
Trial:	L100392		128	Cauliflower	Whole plant[2]	<0.01	<0.01	<0.01	<0.01	-
	Germany		144		Inflorescence	<0.01	<0.01	<0.01	<0.01	-
Study code:	370493				**31 day plant back interval**					
DocID:	2012/1321579	BAS 610 06 F	65	Spinach	Whole plant	<0.01	<0.01	<0.01	<0.01	-
GLP:	Yes	1 x 4.05	73		Leaves	<0.01	<0.01	<0.01	<0.01	-
Year:	2011	to bare soil								
Trial:	L100719									
	Germany									
Study code:	370493				**31 day plant back interval**					
DocID:	2012/1321579	BAS 610 06 F	85	Carrot	Whole plant[1]	<0.01	0.015	<0.01	<0.01	-
GLP:	Yes	1 x 4.05	121		Roots	<0.01	<0.01	<0.01	<0.01	<0.01
Year:	2010	to bare soil	121		Tops	<0.01	<0.01	<0.01	<0.01	-
Trial:	L100394		107	Lettuce	Leaves	<0.01	<0.01	<0.01	<0.01	-
	France (S)		114		Head	<0.01	<0.01	<0.01	<0.01	-
			130	Cauliflower	Whole plant[2]	<0.01	<0.01	<0.01	<0.01	-
			143		Inflorescence	<0.01	<0.01	<0.01	<0.01	-
Study code:	370493				**33 day plant back interval**					
DocID:	2012/1321579	BAS 610 06 F	102	Carrot	Whole plant[1]	<0.01	0.0635	<0.01	<0.01	-
GLP:	Yes	1 x 4.05	151		Roots	<0.01	<0.01	<0.01	<0.01	<0.01
Year:	2010	to bare soil	151		Tops	<0.01	0.017	<0.01	<0.01	-
Trial:	L100395		89	Lettuce	Leaves	<0.01	<0.01	<0.01	<0.01	-
	Spain		102		Head	<0.01	<0.01	<0.01	<0.01	-
			151	Cauliflower	Whole plant[2]	<0.01	<0.01	<0.01	<0.01	-
			179		Inflorescence	<0.01	<0.01	<0.01	<0.01	-
Study code:	370493				**30 day plant back interval**					
DocID:	2012/1321579	BAS 610 06 F	93	Carrot	Whole plant[1]	<0.01	<0.01	<0.01	<0.01	-
GLP:	Yes	1 x 4.05	134		Roots	<0.01	<0.01	<0.01	<0.01	<0.01
Year:	2010	to bare soil	134		Tops	<0.01	<0.01	<0.01	<0.01	-
Trial:	L100599		79	Lettuce	Leaves	<0.01	<0.01	<0.01	<0.01	-
	Belgium		99		Head	<0.01	<0.01	<0.01	<0.01	-
			113	Cauliflower	Whole plant[2]	<0.01	<0.01	<0.01	<0.01	-
			134		Inflorescence	<0.01	<0.01	<0.01	<0.01	-

1 With roots
2 Without roots
I RP30228 (Reg. No 5079647), expressed as parent equivalent. The conversion factor to parent is 0.9999.
II RP32490 (Reg. No 5079628), expressed as parent equivalent. The conversion factor to parent is 1.143.
III RP32596 (Reg. No 85831)
IV M610F007 (Reg. No 5916256) , for details see CA 6.3.1/6

Overall summary rotational crop

The data available from rotational crop metabolism studies indicate that the same reaction pathways are pursued as in target crops: the residues found in follow crops mainly consist of parent iprodione accompanied by its main metabolites RP30228 and RP32490. Beyond, metabolites RP30112, RP30221 and RP25040 are identified as minor compounds. Conjugation to naturally occurring amino acids and carbohydrates as preferred reaction of free amino groups of urea and biuret units is responsible for considerable amounts of bound residues. All these observations are in accordance with the findings after foliar application, only the degree of metabolization is slightly higher in follow crops.

Since the use pattern applied in the formerly performed field rotational crop studies was very different from the uses intended at present, a new field study was performed investigating the residue levels in vegetable follow crops with close rotations with the following result:

In follow crops like spinach, lettuce, carrot and cauliflower planted on iprodione treated soil at a rate of 1x 4 kg as/ha after a re-plant interval of 30 days, no residues of iprodione or its metabolites RP30228 and RP32490 were found above LOQ in edible plant parts. Only metabolite RP30228 was detected up to 0.06 mg/kg in carrot whole plant and up to 0.017 mg/kg in carrot tops. Also 3,5-dichloroaniline (RP32596) was not found above the LOQ of 0.01 mg/kg in any of the samples. Finally, its conjugate with glutamic acid, M610F007 which was identified as typical metabolite in roots of treated carrots was not found above LOQ in carrot as follow crop.

It was shown that the application of iprodione at a rate of 4 kg as/ha does not lead to any quantifiable residues in edible parts of vegetables with short development times even when planted after a short re-plant interval of 30 days. This scenario can be regarded as worst case situation and it can therefore be concluded that after longer waiting periods or in plants with longer development times no residues of iprodione or its metabolites will be found.

CA 6.7 Proposed residue definitions and maximum residue levels

CA 6.7.1 Proposed residue definitions

Plant Matrices

For proposing a suitable residue definition in plant and animal matrices, multiple investigations were performed. As presented in sections 6.2, 6.3, 6.5 and 6.6, plant and animal studies were performed in which iprodione was applied according to the intended use patterns.

For deriving a suitable **residue definition for food of plant origin**, the following studies were considered:

- Crop metabolism studies in five different crop categories
- Confined rotational crop studies
- New supervised field trials with separate analysis for metabolites
- Hydrolysis at exaggerated temperatures simulating processing

Six **metabolism** studies were performed using ^{14}C-phenyl-labelled iprodione covering the categories of fruit crops, leafy vegetables, cereals, pulses/oilseeds and root crops. Confined rotational crop studies were additionally conducted investigating representative crops at different replant intervals after application of iprodione to bare soil. Indications for a deviating reaction pathway in follow crops were not found.

In general, metabolism of iprodione in plants comprises

- cleavage of the dioxoimidazolidine ring followed by isomerization to form RP30228
- desalkylation under formation of RP32490
- further stepwise degradation of both possible heterocyclic rings / the biuret unit
- incorporation into carbohydrates by conjugation of the different urea derivatives.

The metabolism studies indicate that parent iprodione is usually the predominant component accompanied by its metabolites RP30228 and RP32490 which were observed up to about 10% of the TRR. Other degradation products identified in plant (RP36112, RP36115, RP36221, RP25040 and RP44247) were found not exceeding 5% of the TRR.

3,5-Dichloroaniline (DCA, RP32596) was not found in plant metabolism studies in free form. However, its conjugate with naturally occurring glutamic acid, M610F007 was identified in the new metabolism study in carrot. This proves the well-known strong tendency of DCA to react with naturally occurring amino acids or carbohydrates.

In addition, **supervised field trials** in the representative crops carrot and lettuce were conducted with separate determination for RP30228, RP32490, RP32596, RP37176 and M610F007. Furthermore, field trials with separate metabolite analysis in additional crops were performed which are not part of this dossier but which are shown as supplementary information in order to support the revised residue definition (CA 6.3.3).

From both metabolism and field studies can be seen that the metabolites RP30228 and RP32490 are found in most plant matrices investigated. Although the amounts are varying in different crops it can be concluded that RP30228 and RP32490 can be realistically rated as 10% each of parent iprodione (CA 6.3.3).

RP37176 and RP32596 were only found very occasionally in lettuce. M610F007 was mainly found in roots. In other crop parts it was identified only in traces below the limit of quantitation. It can be understood as follow molecule of minor amounts of DCA being temporarily formed.

To simulate processing, **hydrolysis studies** were performed at exaggerated temperatures. Radiolabelled iprodione was incubated under the conditions representative of boiling, baking, brewing, sterilisation and pasteurisation.

The studies investigating the nature of the residues during processing lead to the result that iprodione proves to be stable at pH 4 and medium temperature (70 or 90°C). At pH 5 at 100°C parent is still major compound but the constitutional isomer RP30228 is increasingly formed together with traces of ring-opened metabolite RP37176 and final degradation product RP32596. At pH 6 and 120°C the ratio of parent and RP30228 decrease again in favour of RP37176 and RP32596.

The quantitative differences between the former and the new study, especially regarding the compound of concern RP32596, can partly be explained by the milder conditions of the latter.

However, the difference in quantity of degradation product RP32596 is irrelevant as these results only apply to the medium of pure solvent without any plant matrix. As seen from the carrot metabolism study as well as from literature, it is known that RP32596 has the strong tendency to form conjugates with naturally occurring amino acids and carbohydrates. Therefore, a quantitative extrapolation from a hydrolysis in pure solvent to processing procedures in plant matrices is not possible.

In chapter CA 6.5 it could be shown that RP30228 is formed during processing. However, the sum of parent and RP30228 is clearly decreasing in comparison to the unprocessed material so that no accumulation of iprodione derived residues must be taken into account. Also RP37176 which was found in the ^{14}C-hyrolysis was formed at considerably lower level in the carrot processing study. Exposure calculations based on this study show that RP37176 must not be taken into consideration.

3,5-dichloroaniline (3,5-DCA, RP 36596) was found in processing studies, however, in lower amounts compared to the hydrolysis in solvent. By means of transfer factors levels in processed plant products were calculated. A risk assessment based on expected residues in process fractions and the highest values found in RAC (lettuce) would lead to an exceedance of the Cramer class II threshold of 1.5 µg/kg bw/d. Therefore, an 28-day-study in rats was performed to derive an ADI for 3,5-DCA. The so found value of 12.5 µg/kg bw/d does well cover the exposure calculated with MRL values as worst case assumption. Based on these results it is not regarded necessary to include 3,5-dichloroaniline (RP32596) into the residue definition.

Based on these findings from all studies summarised above the following residue definitions for iprodione in plant matrices are proposed:

For dietary risk assessment:	**Iprodione x conversion factor 1.2** (accounting 10% each for RP30228 and RP32490)
For MRL setting	**Iprodione**

Animal matrices

The general metabolic pathway in rodents and ruminants was found to be comparable: Ensuing from the degradation routes as explained for plant metabolism, oxidation reactions are observed mainly at the isopropyl group and/or at the phenyl ring leading to a number of hydroxylated and carboxylic derivatives. Altogether, the compounds can be divided into one group of metabolites containing the 3,5-dichloroaniline moiety and a second group containing the hydroxy-3,5-dichloroaniline group. The predominant metabolite in most matrices is RP 32490 followed by parent iprodione which mostly accounts for less than 5% of the total extractable residue. In addition, a number of other metabolites is identified.

Based on these data from the metabolism studies it was formerly decided to define the relevant residues for both data generation and enforcement as “sum of iprodione and all metabolites containing the 3,5-dichloroaniline moiety”. For analysis of the so defined relevant residue a common moiety method was necessary although this concept bears the serious disadvantage of not being specific as 3,5-DCA is a also metabolite of both procymidone and vinclozolin. Although inclusions on Annex I for these compounds are expired and authorisations are withdrawn it would still be very valuable to have a method at command which allows differentiation and specific analysis, especially in cases of misuse. Therefore, it should be preferred to use a compound specific marker molecule occurring in all matrices. In case of iprodione the main metabolite in animal matrices RP32490 can be used for this purpose. The potential objection that the ratio of RP32490 in some matrices may be too low can be refuted by using an enforcement method with a limit of quantitation of 0.01 mg/kg which allows the detection of a residue in the same order of magnitude as in the past.

In the 'Reasoned opinion on the review of the existing maximum residue levels (MRLs) for iprodione according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2013;11(10):3438, 94 pp. doi:10.2903/j.efsa.2013.3438' it was stated that the contribution of the hydroxylated metabolites in the matrices milk and kidney was not sufficiently taken into account. In order to avoid the necessity of a second method for enforcement purposes it is proposed to use adequate conversion factors.

As a consequence of these summarized facts and reasons it is proposed to define the relevant residues of iprodione in animal matrices as follows:

For risk assessment

in milk and kidney:	**Sum of iprodione and all metabolites containing the 3,5-dichloroaniline or 3,5-dichloro-4-hydroxyaniline moieties, expressed as iprodione**
in other animal matrices:	**Sum of iprodione and all metabolites containing the 3,5-dichloroaniline moiety, expressed as iprodione**
For enforcement	**RP 32490, expressed as iprodione**

To demonstrate the suitability of RP32490 as specific marker molecule to monitor residues of iprodione in animal matrices, the results from the metabolism studies are summarized in Table 6.7.1-1 together with the conversion factors proposed for risk assessment:

Table 6.7.1-1: Results of metabolism studies as basis for residue definition for risk assessment

Matrix	% TRR from cow, goat and hen metabolism studies			Conversion factor	Residue definition for Risk Assessment
	non-hydroxy-Metabolites	Hydroxy-Metabolites	RP 32490		
Cow milk	23.6	38.8	10	6	Parent + non-hydroxy + hydroxy Metabolites
Goat kidney	27.0	28.0	11.8		
Goat liver	43.7	11.3	19.6	2	Parent + non-hydroxy Metabolites
Goat muscle	43.5	5.3	35.6	1	
Goat fat	84.4	1.7	68	1	
Hen egg	92.6	2.2	36.8	2	
Hen liver	84.3	2.5	22.4	3	
Hen muscle	88.3	1.2	74.2	1	
Hen fat	97.1	0.5	62.6	1.5	

Metabolites

In order to address the relevance of the main plant metabolites, the approach as laid down in the Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment, (EFSA Journal 2012;10(07): 2799) was mainly followed.
For the main metabolites RP 30228 and RP 32490 the exposure assessment showed that the Cramer class III trigger of 1.5 µg/kg bw/day was exceeded (see MCA, chapter 6.9). Thus, the two compounds were included into the residue definition for risk assessment by a conversion factor.
Since the exposure assessment showed for all other metabolites to be above the TTC threshold for genotoxicity of 0.0025 µg/kg bw /d, genotoxicity testing is provided for one representative of each metabolite group. Grouping and testing is further described in chapter 5.8.
Additionally, since 3,5-dichloroaniline is a molecule of special concern, a 28-day study in rats was performed with this metabolite as well (see also MCA, chapter 5.8.). The exposure assessment for all metabolites is found in MCA, chapter 6.9.

CA 6.7.2 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed

The following table shows the existing EU MRLs, the tentative MRLs that have recently been published by EFSA according to Article 12(2) of Regulation (EC) No 396/2005 as well as the MRLs proposed in this dossier:

Code Number	Commodity	Existing EU MRL (mg/kg)	MRL [Art. 12][#] proposed by EFSA (mg/kg)	New proposals by BASF (mg/kg)
Enforcement residue definition:		**Iprodione**		
213020	Carrots	0.5	10	0.8
251010	Lambs lettuce	10	20	30
251020	Lettuce	10	25	30
Enforcement residue definition:		**Vinclozolin, Iprodione, Procymidone, sum of all compounds and all metabolites containing the 3,5-dichloroaniline moiety expressed as 3,5-dichloroaniline**	**sum of iprodione and all metabolites containing the 3,5-dichloroaniline moiety expressed as iprodione**	**RP 32490 expressed as iprodione**
1011010	Swine muscle	0.05[a]	0.1[a]	0.01[a]
1011020	Swine fat (free of lean meat)	0.05[a]	0.1[a]	0.01[a]
1011030	Swine liver	0.05[a]	0.1[a]	0.01[a]
1011040	Swine kidney	0.05[a]	0.1[a]	0.01[a]
1012010	Bovine muscle	0.05[a]	0.1[a]	0.01[a]
1012020	Bovine fat	0.05[a]	0.1[a]	0.01[a]
1012030	Bovine liver	0.05[a]	0.2	0.01[a]
1012040	Bovine kidney	0.05[a]	0.3	0.015
1013010	Sheep muscle	0.05[a]	0.1[a]	0.01[a]
1013020	Sheep fat	0.05[a]	0.1[a]	0.01[a]
1013030	Sheep liver	0.05[a]	0.2	0.01[a]
1013040	Sheep kidney	0.05[a]	0.3	0.015
1014010	Goat muscle	0.05[a]	0.1[a]	0.01[a]
1014020	Goat fat	0.05[a]	0.1[a]	0.01[a]
1014030	Goat liver	0.05[a]	0.2	0.01[a]
1014040	Goat kidney	0.05[a]	0.3	0.015
1016010	Poultry muscle	0.05[a]	0.1[a]	0.01[a]
1016020	Poultry fat	0.05[a]	0.2	0.01[a]
1016030	Poultry liver	0.05[a]	1.0	0.01[a]
1020010	Cattle milk	0.05[a]	0.1	0.01[a]
1020020	Sheep milk	0.05[a]	0.1	0.01[a]
1020030	Goat milk	0.05[a]	0.1	0.01[a]
1030000	Birds' eggs	0.058	0.1	0.01[a]

according to 'Reasoned opinion on the review of the existing maximum residue levels (MRLs) for iprodione according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2013;11(10):3438, 94 pp. doi:10.2903/j.efsa.2013.3438'

a indicates the lower limit of analytical determination

MRLs in this dossier are based on residue trials as described in chapter CA 6.3 with application rates according to the current cGAPs. MRLs are proposed based on the rounded MRLs of parent iprodione derived with the OECD calculator (OECD calculator spreadsheet: http://www.oecd.org/document/34/0,3746,en_2649_37465_48447010_1_1_1_37465,00.html).

Carrot

For carrot 34 trials are available which were performed according to the cGAP (3-4 appl. at 750 g/ha, PHI 27 days). 17 of these trials were done in EU North, 17 in EU South. 8 of the Northern trials were performed in 1995/96 and were already peer reviewed and are therefore not included in this dossier. All other trials done during 1998 and 2011 were described in chapter CA6.3.

0.089, 2x 0.11, 0.12, 2x 0.16, 0.18, 0.23	Northern Europe, already peer reviewed
0.05, 0.07, 2x 0.08, 2x 0.09, 0.12, 0.07, 0.20	Northern Europe
0.035, 0.04, 2x 0.11, 0.12, 3x 0.15, 0.16, 0.23, 0.27, 0.30, 0.31, 0.35, 0.40, 0.45, 0.46	Southern Europe

Table 6.7.2-1: MRL calculation for carrot

OECD Calculator	Iprodione [mg/kg]		
	N-EU	**S-EU**	**N + S**
Highest residue	0.230	0.460	0.460
Mean + 4 SD	0.324	0.769	0.625
CF x 3 Mean	0.374	0.669	0.522
Rounded MRL	**0.4**	**0.8**	**0.7**
STMR	0.11	0.16	0.15

Based on these calculations the following EU MRL is proposed according to the residue definition for enforcement (parent iprodione) and the present residue data:

0.8 mg/kg for carrot
0.8 mg/kg the whole group of other root and tuber vegetables except sugar beet (group 0213000)

Lettuce

For lettuce 35 trials are available which were performed according to the cGAP (3-4 appl. at 750 g/ha). 12 of these trials were done outdoor in EU North, out of which 3 were done between 1974 and 1977 and are already peer reviewed and are therefore not included in chapter CA6.3. In EU South, 10 outdoor trials were conducted. Finally, 13 indoor trials are available which were done according to the cGAP (2 already evaluated).

< 0.03, < 0.05, 8.8	Northern Europe, already peer reviewed
<0.01, 0.025, 0.01, 0.04, 0.05, 0.06, 0.08, 0.48, 1.56	Northern Europe
2x <0.01, 0.02, 0.04, 0.06, 0.17, 0.22, 0.23, 0.24, 0.30	Southern Europe
3.6, 7.2	Indoor (N), already peer reviewed
0.53, 2.5, 3.4, 4.1, 4.6, 7.8, 8.1, 11, 14, 2x 15	Indoor (N+S)

Table 6.7.2-2: MRL calculation for lettuce

OECD Calculator	Iprodione [mg/kg]			
	N-EU, outdoor	**S-EU, outdoor**	**indoor**	**all**
Highest residue	8.800	0.300	15.000	15.000
Mean + 4 SD	11.000	0.582	27.216	21.951
CF x 3 Mean	2.331	0.337	22.345	8.478
Rounded MRL	**15**	**0.6**	**30**	**30**
STMR	0.051	0.115	7.2	0.24

Based on these calculations an EU MRL of

30 mg/kg for lettuce and salad plants (group 0251000)

is proposed according to the residue definition for enforcement (parent iprodione) and the present residue data.

Animal matrices

Feed burden calculations

In the 'Reasoned opinion on the review of the existing maximum residue levels (MRLs) for iprodione according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2013;11(10):3438, 94 pp. doi:10.2903/j.efsa.2013.3438' a chronic consumer risk assessment on the basis of the existing and newly proposed EU MRLs is calculated which consequently also comprises a feed burden calculation based on these values.

Intended uses with the representative formulation BAS 610 06 F covered in this submission include the crops carrot and lettuce. These crops are not relevant as feed items.

In this submission, also a risk assessment describing the uses intended by BASF in future is enclosed to provide a realistic overview beyond the representative uses. Therefore, also a new feed burden calculation based on these data is necessary.

In contrast to the feed burden calculation performed by EFSA, the feed burden covering the future uses comprises less crops:

Cabbage which was identified as major contributor will not be defended in future. In case of lemon, only a post-harvest use exists. It can be assumed that lemons which were treated post-harvest and kept in refrigerated storage are only used as table fruit and not for commercial juice production. Therefore, inclusion of residues in lemon pomace derived from a post-harvest use is not justified. The same applies to pome fruit. Also in this case, the costly post-harvest treatment and cold storage would only be used for table fruit. Therefore, in case of pome fruit, a median value derived from the less critical foliar use is taken for the feed burden calculation.

The input values used for this calculation are summarized in Table 6.7.2-3

Table 6.7.2-3: Input values used for feed burden calculation

Commodity	Median dietary burden		Maximum dietary burden	
Residue definition for risk assessment: Iprodione x conversion factor 1.2				
apple (foliar use) [a]	0.34	median x 1.2	0.36	highest residue x 1.2
peas (dry)	0.03	median x 1.2	0.12	highest residue x 1.2
beans (dry)	0.07	median x 1.2	0.11	highest residue x 1.2
rape seed	0.18	median x 1.2	0.72	highest residue x 1.2

a EFSA Journal 2013;11(10):3438

The results of the calculations are reported in Table 6.7.2-4

Table 6.7.2-4: Results of the feed burden calculation

Animal species	Median dietary burden (mg/kg bw per d)	Maximum dietary burden (mg/kg bw per d)	Highest contributing commodity	Max dietary burden (mg/kg DM)	Trigger exceeded (Y/N)
Residue definition for risk assessment: Iprodione x conversion factor 1.2					
swine	0.003	0.003	rape seed	0.074	N
meat ruminants	0.051	0.054	apple pomace	1.25	Y
poultry	0.003	0.003	beans, dry	0.045	N
dairy ruminants	0.016	0.017	apple pomace	0.470	Y

In the following it is described which residues are to be expected based on (1) the results of the metabolism studies in livestock (2) the feeding studies as well as (3) the feed burden as shown above.

Table 6.7.2-5: Expected residues in animal matrices

a	b	c	d	e	f	g	h	i	j	k	l	m
Matrix	**% TRR from metab. studies in cow, goat, hen**			**Results of feeding study**		**Feed burden (mg/kg bw/d)**		**Expected residues / MRL proposal expressed as iprodione (mg/kg) #**				
	non-hydr.-metab.	**hydr.-metab.**	**RP 32490**	**dose level (mg/kg bw/d)**	**residue (mg/kg)**	**med.**	**max.**	**non-hydr. metabolite moiety**		**RP32490**		**MRL Proposal for residue def.: RP32490**
								med.	**max.**	**med.**	**max.**	
Swine muscle	43.5	5.3	35.6	1.81	0.07	0.003	0.003	<	<	<	<	0.01
Swine fat	84.4	1.7	68	0.54	0.05	0.003	0.003	<	<	<	<	0.01
Swine liver	43.7	11.3	19.6	0.54	0.13	0.003	0.003	<	<	<	<	0.01
Swine kidney	27	28	11.8	0.54	0.16	0.003	0.003	<	<	<	<	0.01
Bov. muscle	43.5	5.3	35.6	1.81	0.07	0.051	0.054	<	<	<	<	0.01
Bovine fat	84.4	1.7	68	0.54	0.05	0.051	0.054	<	<	<	<	0.01
Bovine liver	43.7	11.3	19.6	0.54	0.13	0.051	0.054	0.012	0.013	<	<	0.01
Bov. kidney	27	28	11.8	0.54	0.16	0.051	0.054	0.015	0.016	0.01	0.01	0.015
Poultry meat	88.3	1.2	74.2	1.5	0.26	0.003	0.003	<	<	<	<	0.01
Poultry fat	97.1	0.5	62.6	0.15	0.15	0.003	0.003	<	<	<	<	0.01
Poultry liver	84.3	2.5	22.4	0.15	0.53	0.003	0.003	0.011	0.011	<	<	0.01
Milk	23.6	38.8	10	0.54	0.041	0.016	0.017	<	<	<	<	0.01
Poultry egg	92.6	2.2	36.8	0.15	0.10	0.003	0.003	<	<	<	<	0.01

To improve readability in column i – l "<" was given for "< LOQ"

Table 6.7.2-5 shows the following:

- the results of the metabolism studies in livestock (in %TRR) are presented as sum of non-hydroxylated metabolites and sum of hydroxylated metabolites (columns b and c), in addition the ratio of metabolite RP32490 which is proposed as marker molecule for enforcement is given separately (column d)
- the dose levels and residues found in the feeding studies reported according to the residue definition of "sum of iprodione and non-hydroxylated metabolites as iprodione" are shown in columns e and f
- the feed burden (column g and h) was calculated using apple (foliar), pulses and oilseed rape as demonstrated above
- based on these data, residues would be expected according to the existing residue definition of "sum of iprodione and non-hydroxylated metabolites as parent" in bovine liver and kidney and in poultry liver (columns i and j)
- using metabolite RP32490 as marker molecule to monitor iprodione residues in animal matrices leads to residues in bovine kidney only. In all other animal matrices residues are expected to be below the LOQ of 0.01 mg/kg

It must be stated that the residues definition as proposed by EFSA (EFSA Journal 2013;11(10):3438) which is "sum of iprodione and non-hydroxylated metabolites as parent" requires the use of a common moiety method which suffers from the disadvantage of non-specificity because it is shared with vinclozolin and procymidone. In addition, it has a high limit of quantitation of 0.1 mg/kg. This means that based on this feed burden the expected residues according to this definition in liver and kidney could also not be quantified.

Therefore, the new proposal to use metabolite RP32490 as marker molecule is considered as justified. Despite the relatively low ratio of this molecule in the different animal matrices the detection of residues can be achieved in the same order of magnitude as with the common moiety method by using a modern and compound-specific analytical method with a low LOQ of 0.01 mg/kg.

In the 'Reasoned opinion on the review of the existing maximum residue levels (MRLs) for iprodione according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2013;11(10):3438, 94 pp. doi:10.2903/j.efsa.2013.3438' a concern was raised regarding residues in animal matrices:

It was criticized that the amounts of hydroxyl-metabolites in milk and kidney, namely the ratio of DCHPU (22% TRR in bovine kidney) and RP36114 in milk (38.1% TRR in milk) are not sufficiently taken into account, especially as no toxicological data were available to EFSA. These deficiencies also led to a request for a new feeding study because the hydroxylated metabolites have not been analysed in the cow feeding study.

In this dossier, the data situation concerning this question is more favourable:

- Based on the feed burden shown above the levels of the expected residues levels in animal matrices are lower
- Compared to the old residue definition for risk assessment, the hydroxylated metabolite moiety is more taken into consideration by the proposed definition and the conversion factor of 6 for bovine kidney and milk
- All iprodione metabolites were assigned to groups out of which representatives were further tested (see CA 5.8)
- For all metabolites an exposure calculation was performed by means of which the relevance was assessed (see CA 6.9). As the two hydroxylated metabolites are only observed in animal matrices the overall exposure is very low. In case of DCHPU an exposure even below the threshold for genotoxic compounds (0.0025 µg/kg bw/day) resulted on the basis of this feed burden.

Against this background a complete new feeding study in order to provide the analysis of one metabolite in one matrix seems not to be justified.

CA 6.7.3 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed for imported products (import tolerance)

Plant Products

According to the proposed residue definition for MRL setting of

Iprodione parent

and referring to MRL derivations in chapter CA 6.7.2 it is proposed to establish EU MRLs of:

0.8 mg/kg **for carrot and the whole group of other root and tuber vegetables except sugar beet (group 0213000)**

30 mg/kg **for lettuce and salad plants (group 0251000)**

Animal products

According to the proposed residue definition for MRL setting of

RP32490 expressed as iprodione

and referring to MRL derivations in chapter CA 6.7.2 it is proposed to establish EU MRLs of:

0.01 mg/kg **for all animal tissues, bird's eggs and milk/cream except bovine kidney**
0.015 mg/kg **for bovine kidney**

CA 6.8 Proposed safety intervals

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 carrot application at the growth stage BBCH 13-49 with the pre-harvest interval of 27 days is intended. For lettuce, application is possible during growth stage BBCH 10-49 with pre-harvest intervals of 21 days (outdoor) and 14 days (indoor).

Re-entry period for livestock to areas to be grazed
Because iprodione 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 treated crops
Treated crops or areas should not be re-entered until leaf or soil surfaces are dry. It is not necessary to determine a particular re-entry time for workers.

Witholding period for animal feed stuffs
The representative uses in this dossier, carrot and lettuce, are not intended as animal feeding stuff.

Waiting period between application and crop sowing or planting the crop to be protected
No waiting period is necessary since iprodione is not intended in pre-emergence emergence use.

Waiting period between application and handling treated produce
This is not relevant here since a post-harvest treatment is not intended for carrot and lettuce.

Waiting period between last application and sowing or planting succeeding crops
Iprodione has shown, over a number of years of use with a range of different formulation types across a very wide range of crop species, to be safe to apply without any reports of adverse effects on following crops. This is of significance as, due to the very diverse range of arable and horticultural crops in which the product has been used, most rotational crop possibilities have been tested. Moreover no waiting period is deemed necessary regarding residues. No relevant accumulation of BAS 610 F or its degradation products were observed in the confined rotational crop study (see document MCA, section 6.6), no limitation concerning the succeeding crops is necessary regarding residues.

CA 6.9 Estimation of the potential and actual exposure through diet and other sources

Acceptable Daily Intake (ADI) and Dietary Exposure Calculation

TMDI calculations

Risk assessments according to newly proposed residue definitions

In the 'Reasoned opinion on the review of the existing maximum residue levels (MRLs) for iprodione according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2013;11(10):3438, 94 pp. doi:10.2903/j.efsa.2013.3438' chronic consumer risk assessments for iprodione were performed in two different ways:
Firstly, a calculation was performed using only EU MRLs (tentative median residues or existing MRLs) without considering the existing CXLs (Scenario 1). In comparison, a second calculation showed the result taking the CXLs into account (Scenario 2). The results are summarized in the following table:

Table 6.9-1: Results of chronic risk assessments performed by EFSA, 2013 #

Input values		Highest exposure	
		% of ADI	Diet
Residue definition for risk assessment:	**iprodione**		
ADI:	**0.06 mg/kg bw/d (Dir 03/V92 and JMPR 2001)**		
Scenario 1:	tentative STMR / MRLs	42.6	DE child
Scenario 2:	tentative STMR / MRLs + CXLs	57.8	DE child

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Though the EU MRL proposals were considered tentative by EFSA in this context it was concluded that an exposure based on these calculations does not indicate a risk to consumers.

As described in chapter CA 6.7 a new residue definition for risk assessment was proposed for plant: Based on metabolism and supervised field trial data it was shown that the two main metabolites in plant matrices, RP30228 and RP32490 should be taken into account by 10% of the parent concentration. Therefore, new risk assessments including these changes were performed. In scenario 3 only the representative crops carrot and lettuce are included, whereas scenario 4 comprises all crops to be defended by BASF in future which can be understood in anticipation of the product re-registration scheduled for 2017.
Please note: the MRL proposals used in scenario 4, other than for carrot and lettuce, are not meant to be evaluated in this dossier. They are used to show the influence of the revised residue definition for risk assessment in an overview.

Table 6.9-2 provides the following:

- existing EU MRLs and CXLs
- tentative MRLs from EFSA together with input values for scenarios 1 and 2
- new MRL proposals from BASF together with input values for scenarios 3 and 4

Table 6.9-2: Existing and proposed MRLs for iprodione in the EU and input values for risk assessments

Code Number	Commodity	Existing EU MRL (mg/kg)	Existing CXL (mg/kg)	EFSA Reasoned Opinion [Art. 12][#]					BASF: Proposals for representative and future uses					
				Prop. MRL (mg/kg)	Input values for chronic RA				MRL (mg/kg)	STMR (mg/kg)	Input values for chronic RA			
					Scenario 1: tentative STMR / MRL		Scenario 2: tentative STMR/MRL + CXL				Scenario 3: Representative uses only		Scenario 4: all crops to be defended in future	
					(mg/kg)	comment	(mg/kg)	comment			(mg/kg)	comment	(mg/kg)	comment
Residue definitions		**Enforcement and Risk assessment: iprodione**							**Enforcement: iprodione**		**Risk assessment: iprodione x 1.2**			
110030	Lemons	5	-	6	3.50	STMR	3.50	STMR	6	3.5	-	-	4.2	STMR x 1.2 b)
120010	Almonds	0.02 [a]	0.2	0.2	0.02	STMR	0.05	STMR CXL	0.02	0.02	-	-	0.024	STMR x 1.2 b)
120060	Hazelnuts	0.2	-	0.2	0.20	MRL a)	0.20	MRL a)	0.3	0.1	-	-	0.12	STMR x 1.2 b)
130010	Apples	5	5	6	0.95	STMR	0.95	STMR	7	1.8	-	-	2.16	STMR x 1.2 b)
130020	Pears	5	5	6	0.95	STMR	0.95	STMR	7	1.8	-	-	2.16	STMR x 1.2 b)
130030	Quinces	5	5	5	-	-	1.70	STMR CXL	7	1.8	-	-	2.16	STMR x 1.2 b)
130040	Medlar	5	5	5	-	-	1.70	STMR CXL	7	1.8	-	-	2.16	STMR x 1.2 b)
130050	Loquat	5	5	5	-	-	1.70	STMR CXL	7	1.8	-	-	2.16	STMR x 1.2 b)
140010	Apricots	3	-	6	1.80	STMR	1.80	STMR	c)	-	-	-	-	-
140020	Cherries	3	10	10	1.35	STMR	1.40	STMR CXL	c)	-	-	-	-	-
140030	Peaches	3	10	10	1.80	STMR	2.90	STMR CXL	5	1.45	-	-	1.74	STMR x 1.2 b)
140040	Plums	3	-	3	0.16	STMR	0.16	STMR	1.5	0.23	-	-	0.28	STMR x 1.2 b)
151000	Table and wine grapes	10	10	20	3.20	STMR	3.20	STMR	15	3.78	-	-	4.54	STMR x 1.2 b)
152000	Strawberries	15	10	20	2.60	STMR	2.60	STMR	15	2.2	-	-	2.64	STMR x 1.2 b)
153010	Blackberries	10	30	30	3.45	STMR	9.45	STMR CXL	15	2.85	-	-	3.42	STMR x 1.2 b)
153020	Dewberries	10	-	15	3.45	STMR	3.45	STMR	15	2.85	-	-	3.42	STMR x 1.2 b)
153030	Raspberries	10	30	30	3.45	STMR	9.45	STMR CXL	15	2.85	-	-	3.42	STMR x 1.2 b)
154010	Blueberries	10	-	20	8.15	STMR	8.15	STMR	15	3.2	-	-	3.84	STMR x 1.2 b)
154030	Currants (red, black, white)	10	-	20	8.15	STMR	8.15	STMR	15	3.2	-	-	3.84	STMR x 1.2 b)
154040	Gooseberries	10	-	20	8.15	STMR	8.15	STMR	15	3.2	-	-	3.84	STMR x 1.2 b)

Table 6.9-2: Existing and proposed MRLs for iprodione in the EU and input values for risk assessments

Code Number	Commodity	Existing EU MRL (mg/kg)	Existing CXL (mg/kg)	EFSA Reasoned Opinion [Art. 12]#					BASF: Proposals for representative and future uses					
				Prop. MRL (mg/kg)	Input values for chronic RA				MRL (mg/kg)	STMR (mg/kg)	Input values for chronic RA			
					Scenario 1: tentative STMR / MRL		Scenario 2: tentative STMR/MRL + CXL				Scenario 3: Representative uses only		Scenario 4: all crops to be defended in future	
					(mg/kg)	comment	(mg/kg)	comment			(mg/kg)	comment	(mg/kg)	comment
Residue definitions		**Enforcement and Risk assessment: iprodione**							**Enforcement: iprodione**		**Risk assessment: iprodione x 1.2**			
162010	Kiwi	5	5	5	1.20	STMR	1.20	STMR	c)	-	-	-	-	-
211000	Potatoes	0.02 [a]	-	0.05	0.05	STMR	0.05	STMR	c)	-	-	-	-	-
213010	Beetroot	0.02 [a]	-	0.1	0.1	STMR	0.1	STMR	c)	-	-	-	-	-
213020	Carrots	0.5	10	10	0.21	STMR	5.60	STMR CXL	0.8	0.16	0.19	STMRx1.2 [b)]	0.19	STMR x 1.2 b)
213000	whole group of other root and tuber veg. except sugar beet	-	-	-	-	-	-	-	0.8	0.16	0.19	STMRx1.2 [b)]	0.19	STMR x 1.2 b)
213030	Celeriac	0.02 [a]	-	0.02	0.02	MRL a)	0.02	MRL a)						
213040	Horseradish	0.5	-	0.06	0.05	STMR	0.05	STMR						
213060	Parsnips	0.5	-	0.5	0.14	STMR	0.14	STMR						
213070	Parsley root	0.5	-	0.5	0.14	STMR	0.14	STMR						
213080	Radishes	0.3	-	0.6	0.18	STMR	0.18	STMR						
231100	Swedes	0.02*	-	0.02	0.02	MRL a)	0.02	MRL a)						
213110	Turnips	0.02*	-	0.02	0.02	MRL a)	0.02	MRL a)						
220010	Garlic	0.2		0.4	0.08	STMR	0.08	STMR	0.2	0.02	-	-	0.024	STMR x 1.2 b)
220020	Onions	0.2	0.2	0.2	0.02	STMR	0.02	STMR	0.2	0.02	-	-	0.024	STMR x 1.2 b)
220030	Shallots	0.2	-	0.2	0.02	STMR	0.02	STMR	0.2	0.02	-	-	0.024	STMR x 1.2 b)
220040	Spring onions	3	-	4	0.78	STMR	0.78	STMR	3	0.64	-	-	0.76	STMR x 1.2 b)
231010	Tomatoes	5	5	5	1.01	STMR	1.10	STMR CXL	4	1	-	-	1.2	STMR x 1.2 b)
231020	Peppers	5	-	7	2.00	STMR	2.00	STMR	8	2	-	-	2.4	STMR x 1.2 b)
231030	Aubergines (egg plants)	5	-	6	1.50	STMR	1.50	STMR	4	1	-	-	1.2	STMR x 1.2 b)
232010	Cucumbers	2	2	4	1.30	STMR	1.30	STMR	3	0.45	-	-	0.54	STMR x 1.2 b)

Table 6.9-2: Existing and proposed MRLs for iprodione in the EU and input values for risk assessments

Code Number	Commodity	Existing EU MRL (mg/kg)	Existing CXL (mg/kg)	EFSA Reasoned Opinion [Art. 12]#					BASF: Proposals for representative and future uses					
				Prop. MRL (mg/kg)	Input values for chronic RA				MRL (mg/kg)	STMR (mg/kg)	Input values for chronic RA			
					Scenario 1: tentative STMR / MRL		Scenario 2: tentative STMR/MRL + CXL				Scenario 3: Representative uses only		Scenario 4: all crops to be defended in future	
					(mg/kg)	comment	(mg/kg)	comment			(mg/kg)	comment	(mg/kg)	comment
Residue definitions		**Enforcement and Risk assessment: iprodione**							**Enforcement: iprodione**		**Risk assessment: iprodione x 1.2**			
232020	Gherkins	2	-	4	1.30	STMR	1.30	STMR	3	0.45	-	-	0.54	STMR x 1.2 b)
232030	Courgettes	2	-	4	1.30	STMR	1.30	STMR	3	0.45	-	-	0.54	STMR x 1.2 b)
233000	Cucurbits w inedible peel	1	-	1.5	0.17	STMR	0.17	STMR	d)	-	-	-	0.2	d)
241010	Broccoli	0.1	25	25	0.05	STMR	25	CXL	0.4	0.02	-	-	0.024	STMR x 1.2 b)
241020	Cauliflower	0.1	-	0.05	0.05	STMR	0.05	STMR	0.4	0.02	-	-	0.024	STMR x 1.2 b)
242010	Brussels sprouts	0.5	-	0.7	0.24	STMR	0.24	STMR	0.7	0.24	-	-	0.28	STMR x 1.2 b)
242020	Head cabbage	5	-	15	0.94	STMR	0.94	STMR	c)	-	-	-	-	-
243010	Chinese cabbage	5	-	0.7	0.05	STMR	0.05	STMR	5	0.21	-	-	0.25	STMR x 1.2 b)
243020	Kale	0.02*	-	0.02	0.02	MRL a)	0.02	MRL a)	-	-	-	-	-	-
244000	Kohlrabi	0.02*	-	0.02	0.02	MRL a)	0.02	MRL a)	-	-	-	-	-	-
251010	Lambs lettuce	10	-	20	4.90	STMR	4.90	STMR	30	0.24	0.29	STMRx1.2 b)	0.29	STMR x 1.2 b)
251020	Lettuce	10	25	25	4.90	STMR	4.80	STMR CXL	30	0.24	0.29	STMRx1.2 b)	0.29	STMR x 1.2 b)
251000	Lettuce and salad plants	-	-	-	-	-	-	-	30	0.24	0.29	STMRx1.2 b)	0.29	STMR x 1.2 b)
251030	Scarole (broad-leaf endive)	10	-	20	4.90	STMR	4.90	STMR						
251040	Cress	10	-	7	0.08	STMR	0.08	STMR						
251050	Land Cress	10	-	10	10	MRL a)	10	MRL a)						
251060	Rocket, Rucola	10	-	20	4.90	STMR	4.90	STMR						
251080	Leaves + sprouts of Brassica spp	10	-	20	4.90	STMR	4.90	STMR						
252010	Spinach	0.02*	-	0.02	0.02	MRL a)	0.02	MRL a)	c)	-	-	-		
252030	Beet leaves (chard)	0.02*	-	0.02	0.02	MRL a)	0.02	MRL a)	-	-	-	-		
255000	Witloof	2	1	3	0.91	STMR	0.91	STMR	3	0.91	-	-	1.09	
256000	Herbs	10	-	20	4.90	STMR	4.90	STMR			-	-		

Table 6.9-2: Existing and proposed MRLs for iprodione in the EU and input values for risk assessments

Code Number	Commodity	Existing EU MRL (mg/kg)	Existing CXL (mg/kg)	EFSA Reasoned Opinion [Art. 12]#					BASF: Proposals for representative and future uses					
				Prop. MRL (mg/kg)	Input values for chronic RA				MRL (mg/kg)	STMR (mg/kg)	Input values for chronic RA			
					Scenario 1: tentative STMR / MRL		Scenario 2: tentative STMR/MRL + CXL				Scenario 3: Representative uses only		Scenario 4: all crops to be defended in future	
					(mg/kg)	comment	(mg/kg)	comment			(mg/kg)	comment	(mg/kg)	comment
Residue definitions		**Enforcement and Risk assessment: iprodione**							**Enforcement: iprodione**		**Risk assessment: iprodione x 1.2**			
260010	Beans (fresh, with pods)	5	2	2	0.13	STMR	0.21	STMR CXL	d)	-	-	-	0.16	d)
260020	Beans (fresh, w/o pods)	0.02*	-	0.08	0.02	STMR	0.02	STMR	-	-	-	-	-	-
260030	Peas (fresh, with pods)	2	-	2	0.48	STMR	0.48	STMR	-	-	-	-	-	-
260040	Peas (fresh, w/o pods)	0.3	-	0.3	0.30	MRL a)	0.30	MRL a)	0.3	0.1	-	-	0.12	STMR x 1.2 b)
270010	Asparagus	0.02*	-	0.02	0.02	MRL a)	0.02	MRL a)	d)	-	-	-	0.02*	d)
270030	Celery	0.02*	-	0.02	0.02	MRL a)	0.02	MRL a)	-	-	-	-	-	-
270040	Fennel	0.02*	-	0.02	0.02	MRL a)	0.02	MRL a)	-	-	-	-	-	-
270060	Leek	0.02*	-	0.02	0.02	MRL a)	0.02	MRL a)	-	-	-	-		
270070	Rhubarb	0.2	-	0.15	0.06	STMR	0.06	STMR	-	-	-	-	-	-
300010	Beans (dry)	0.2	0.1	0.4	0.08	STMR	0.08	STMR	0.2	0.06	-	-	0.07	STMR x 1.2 b)
300020	Lentils (dry)	0.2	-	0.4	0.08	STMR	0.08	STMR	0.2	0.06	-	-	0.07	STMR x 1.2 b)
300030	Peas (dry)	0.2	-	0.2	0.03	STMR	0.03	STMR	0.2	0.025	-	-	0.03	STMR x 1.2 b)
401010	Linseed	0.5	-	0.06	0.02	STMR	0.02	STMR			-	-		
401060	Rape seed	0.5	0.5	0.5	0.5	MRL a)	0.5	MRL a)	0.6	0.16	-	-	0.18	
401050	Sunflower seed	0.5	0.5	0.5	-	-	0.07	STMR CXL			-	-		
500090	Wheat grain	0.5	-	0.02	0.02	STMR	0.02	STMR	c)	-	-	-		
500010	Barley grain	0.5	2	2	0.02	STMR	0.40	STMR CXL	c)	-	-	-		
500060	Rice grain	3	10	10	0.87	STMR	0.82	STMR CXL	3	0.9	-	-	1.0	STMR x 1.2 b)
630000	Herbal infusions	0.1 [a]	-	0.1	0.55	STMR	0.55	STMR	c)	-	-	-	-	-
810000	Spices (seeds)	0.1 [a]	0.05*	0.1	0.10	MRL a)	0.10	MRL a)	c)	-	-	-	-	-
820000	Spices (fruits and berries)	2 (caraway)	-	2	2.00	MRL a)	2.00	MRL a)	c)	-	-	-	-	-
840000	Spices (roots and rhizome)	0.1 [a]	0.1*	0.1	0.10	MRL a)	0.10	MRL a)	c)	-	-	-	-	-
900010	Sugar beet (root)	0.2	0.1*	0.1	0.10	STMR	0.10	STMR	c)	-	-	-	-	-

Table 6.9-2 continued: Existing and proposed MRLs for iprodione in the EU and input values for risk assessment

Code Number	Commodity	Existing EU MRL (mg/kg)	EFSA Reasoned Opinion [Art. 12]#			BASF: Proposals for representative and future uses			
			Prop. MRL (mg/kg)	Input values for chronic RA		MRL (mg/kg)	median (mg/kg)	Input values for chronic RA	
				Scenario 1+2	comment			Scenario 4	comment
Residue definitions:		**Enforcem. and RA**	**Enforcement and RA**			**Enforcement**		**Risk assessment**	
		Sum of Vinclozolin, Iprodione, Procymidone and all metabolites contain. the 3,5-DCA moiety expr. as 3,5-DCA	**Sum of iprodione and all metabolites containing the 3,5-DCA moiety expressed as iprodione**			**RP 32490 expressed as iprodione**		**Sum of iprodione and all metabolites containing the 3,5-DCA moiety expressed as iprodione**	
1011010	Swine muscle	0.05[a]	0.1*	0.1*	median	0.01*	0.0001	0.0001	median
1011020	Swine fat (free of lean meat)	0.05[a]	0.1*	0.1*	median	0.01*	0.0002	0.0002	median
1011030	Swine liver	0.05[a]	0.1*	0.1*	median	0.01*	0.0003	0.0006	median x 2
1012010	Bovine muscle	0.05[a]	0.1*	0.1*	median	0.01*	0.0016	0.0016	median
1012020	Bovine fat	0.05[a]	0.1*	0.1*	median	0.01*	0.0038	0.0038	median
1012030	Bovine liver	0.05[a]	0.2	0.12	median	0.01*	0.0055	0.0110	median x 2
1016010	Poultry muscle	0.05[a]	0.1*	0.1*	median	0.01*	0.0004	0.0004	median
1016020	Poultry fat	0.05[a]	0.2	0.1*	median	0.01*	0.0019	0.0029	median x 1.5
1016030	Poultry liver	0.05[a]	1.0	0.11	median	0.01*	0.0028	0.0084	median x 3
1030000	Birds' eggs	0.058	0.1	0.02	median	0.01*	0.0008	0.0016	median x 2
Residue definitions:			**Sum of iprodione and all metab. cont. the 3,5-dichloroaniline or 3,5-dichloro-4-hydroxyaniline moieties, expressed as iprodione**			**RP 32490 expressed as iprodione**		**Sum of iprodione and all metab. cont. the 3,5-dichloroaniline or 3,5-dichloro-4-hydroxyaniline moieties, expressed as iprodione**	
1011040	Swine kidney	0.05[a]	0.1*	0.30	median x 3	0.01*	0.0004	0.0023	median x 6
1012040	Bovine kidney	0.05[a]	0.3	0.45	median x 3	0.015	0.0066	0.0396	median x 6
1020010	Cattle milk	0.05[a]	0.1	0.02	median x 2	0.01*	0.0005	0.0031	median x 6

according to 'Reasoned opinion on the review of the existing maximum residue levels (MRLs) for iprodione according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2013;11(10):3438, 94 pp. doi:10.2903/j.efsa.2013.3438'
* indicates the lower limit of analytical determination
a not supported by data, therefore existing EU MRL used
b STMR x conversion factor 1.2
c use not defended by BASF in future
d new trials ongoing, therefore, EFSA STMR x factor 1.2 was taken

Based on the input values as shown in the above table the following results for the chronic risk assessments are obtained:

Scenario 3 shows that considering only the representative uses as submitted in this dossier would lead to an utilisation of the ADI of below 1 %. This result is does not have a realistic importance and is therefore included for completeness.

Scenario 4 shows the chronic risk assessment including those crops which are to be defended in future. Compared to the calculation performed by EFSA (see above) the number of crops was reduced. Due to the revised residue definition for risk assessment now including a contribution of 10% each of the two main metabolites RP30228 and RP32490 the ADI of 0.06 mg/kg bw/d is utilised up to 65.3% with the German child as highest exposed consumer group.

Table 6.9-3: Results of chronic risk assessments

Input values		Highest exposure	
		% of ADI	Diet
Residue definition for risk assessment:	**iprodione x conversion factor 1.2** (accounting 10% each for RP30228 and RP32490)		
ADI:	**0.06 mg/kg bw/d (Dir 03/V92 and JMPR 2001)**		
Scenario 3: Representative uses only		0.9%	FR toddler
Scenario 4: all crops to be defended in future		65.3%	DE child

The results show that the integration of the metabolites RP30228 and RP32490 into the residue definition for risk assessment does not lead to an increased risk for public health.

Table 6.9-4: TMDI calculation for iprodione with PRIMo Model (rev 2.0) using MRLs for the representative uses submitted in this dossier (Scenario 3)

Iprodione			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):	0.01	proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0.06	ARfD (mg/kg bw):	n.n.
Source of ADI:	JMPR 2001	Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Prepare workbook for refined calculations

Undo refined calculations

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
0 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)
0.9	FR toddler	0.7	Carrots	0.1	Turnips	0.0	Celeriac	
0.9	FR infant	0.8	Carrots	0.1	Turnips	0.0	Celeriac	
0.6	SE general population 90th percentile	0.3	Carrots	0.2	Lettuce and other salad plants	0.0	Beetroot	
0.6	IE adult	0.2	Parsnips	0.1	Carrots	0.1	Swedes	
0.5	WHO Cluster diet B	0.2	Beetroot	0.2	Lettuce and other salad plants	0.1	Carrots	
0.5	DK child	0.4	Carrots	0.1	Lettuce and other salad plants	0.0	Celeriac	
0.4	UK Infant	0.4	Carrots	0.0	Swedes	0.0	Parsnips	
0.4	WHO Cluster diet F	0.1	Lettuce and other salad plants	0.1	Carrots	0.1	Swedes	
0.4	DE child	0.3	Carrots	0.0	Lettuce and other salad plants	0.0	Herbs	
0.4	WHO regional European diet	0.2	Lettuce and other salad plants	0.1	Carrots	0.0	Herbs	
0.4	NL child	0.2	Carrots	0.1	Lettuce and other salad plants	0.1	Beetroot	
0.3	IT adult	0.3	Lettuce and other salad plants	0.0	Carrots	0.0	Herbs	
0.3	ES adult	0.3	Lettuce and other salad plants	0.0	Carrots	0.0	Other other root and tuber	
0.3	WHO cluster diet E	0.1	Carrots	0.1	Lettuce and other salad plants	0.0	Beetroot	
0.3	FR all population	0.2	Lettuce and other salad plants	0.1	Carrots	0.0	Radishes	
0.3	IT kids/toddler	0.2	Lettuce and other salad plants	0.1	Carrots	0.0	Herbs	
0.3	ES child	0.2	Lettuce and other salad plants	0.1	Carrots	0.0	Other other root and tuber	
0.3	PT General population	0.2	Carrots	0.1	Lettuce and other salad plants	0.0	Turnips	
0.2	NL general	0.1	Lettuce and other salad plants	0.1	Carrots	0.0	Beetroot	
0.2	DK adult	0.1	Carrots	0.1	Lettuce and other salad plants	0.0	Celeriac	
0.2	PL general population	0.1	Carrots	0.1	Beetroot	0.0	Parsley root	
0.2	UK Toddler	0.2	Carrots	0.0	Swedes	0.0	Lettuce and other salad plants	
0.2	WHO cluster diet D	0.1	Carrots	0.0	Herbs	0.0	Radishes	
0.2	UK vegetarian	0.1	Lettuce and other salad plants	0.1	Carrots	0.0	Beetroot	
0.1	UK Adult	0.1	Lettuce and other salad plants	0.1	Carrots	0.0	Swedes	
0.1	LT adult	0.1	Carrots	0.0	Beetroot	0.0	Lettuce and other salad plants	
0.1	FI adult	0.1	Carrots	0.0	Lettuce and other salad plants	0.0	Beetroot	

Conclusion:
The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of Iprodione is unlikely to present a public health concern.

Table 6.9-5: TMDI calculation for iprodione with PRIMo Model (rev 2.0) using MRLs for the uses to be defended in future (Scenario 4)

Iprodione			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):	0.01	proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0.06	ARfD (mg/kg bw):	n.n.
Source of ADI:	JMPR 2001	Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Prepare workbook for refined calculations

Undo refined calculations

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	
7	65
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)
65.3	DE child	43.4	Apples	9.6	Table and wine grapes	2.2	Pears	0.1
37.9	NL child	22.8	Apples	5.8	Table and wine grapes	1.5	Pears	0.2
37.2	WHO Cluster diet B	16.2	Table and wine grapes	6.2	Tomatoes	3.6	Apples	0.0
36.7	FR all population	31.1	Table and wine grapes	1.7	Apples	0.9	Tomatoes	0.0
31.8	PT General population	20.9	Table and wine grapes	3.8	Apples	1.8	Tomatoes	
24.4	IE adult	8.8	Table and wine grapes	3.0	Apples	2.4	Pears	0.0
21.6	WHO cluster diet E	13.6	Table and wine grapes	3.0	Apples	1.1	Tomatoes	0.0
19.0	FR toddler	9.4	Apples	2.7	Strawberries	1.6	Table and wine grapes	0.2
17.4	DK child	8.4	Apples	2.4	Pears	1.5	Cucumbers	0.1
17.4	DK adult	11.1	Table and wine grapes	2.8	Apples	0.8	Tomatoes	0.0
15.7	FR infant	9.0	Apples	2.2	Strawberries	1.1	Pears	0.1
14.8	NL general	6.5	Table and wine grapes	4.3	Apples	0.9	Tomatoes	0.0
14.4	UK Toddler	6.1	Apples	2.1	Table and wine grapes	1.2	Tomatoes	0.1
14.3	PL general population	7.4	Apples	2.4	Table and wine grapes	1.8	Tomatoes	
13.6	SE general population 90th percentile	3.8	Apples	1.7	Table and wine grapes	1.5	Tomatoes	0.1
12.8	UK Adult	8.6	Table and wine grapes	1.5	Apples	0.9	Tomatoes	0.0
12.7	WHO cluster diet D	4.2	Table and wine grapes	2.4	Apples	2.0	Tomatoes	0.0
12.6	UK vegetarian	6.7	Table and wine grapes	2.1	Apples	1.2	Tomatoes	0.0
12.3	WHO Cluster diet F	5.5	Table and wine grapes	2.4	Apples	1.4	Tomatoes	0.0
11.8	WHO regional European diet	2.9	Table and wine grapes	2.4	Apples	2.2	Tomatoes	0.0
11.7	ES adult	3.5	Table and wine grapes	2.8	Apples	1.6	Tomatoes	0.0
11.5	UK Infant	5.6	Apples	1.1	Rice	1.0	Strawberries	0.2
11.3	IT kids/toddler	3.2	Apples	2.9	Tomatoes	1.2	Pears	
11.0	ES child	4.1	Apples	2.0	Tomatoes	1.6	Pears	0.1
10.1	IT adult	2.9	Apples	2.3	Tomatoes	1.1	Peaches	
10.1	LT adult	6.7	Apples	1.2	Tomatoes	0.6	Pears	0.0
6.8	FI adult	2.5	Table and wine grapes	1.5	Apples	0.9	Tomatoes	0.0

Conclusion:
The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of Iprodione is unlikely to present a public health concern.

Exposure calculation for metabolites

As described in detail in chapters CA 6.2 to 6.6 a number of iprodione metabolites were identified in matrices directly or indirectly related to human consumption. The following 12 compounds have to be assessed: RP 30228, RP 32490, RP 32596 (3,5-dichloroaniline), RP 36112, RP 25040, RP 36221, RP 36115, RP 36114, RP 44247, DCHPU, M610F007, RP 37176.

From this list of metabolites three compounds, namely RP 32490, RP 36115 and RP 36114, were found to be major metabolites in rats occurring in excreta and appearing to be end products of a chain of biotransformation mechanisms (see chapter CA 5.1). Further evidence for these assumed pathways gives the finding of other metabolites found in smaller amounts and occurring as intermediates. Amongst these are RP 30228 and RP 32112.

Regardless the occurrence in rat metabolism exposure calculations have been performed for all metabolites mentioned above as basis for an assessment using the TTC concept.

These calculations were done using the EFSA PRIMo calculator and considering all uses to be defended in future. In case of metabolites observed mainly in processed commodities the German VELS model was used instead.

In the following tables, the input values used for these calculations as well as the results are shown.

RP 30228

Metabolite RP 30228 is the constitutional isomer of parent iprodione which is found in most plants. As shown in CA 6.3.3 the compound can be realistically estimated with 10% of parent. It was also detected in animal matrices at low level. These assumptions lead to an exposure of 3.3 µg/kg bw/d which is above the trigger of 1.5 µg/kg bw/d.

Metabolite RP 30228 was found to be a minor component in rat metabolism (see CA 5.1) and was evaluated to be less toxic than parent iprodione (see CA 5.8) based on a number of studies.

For that reason, the compound is included into the risk assessment for iprodione by 10%.

Table 6.9-6: Residue values used for the exposure assessment of RP 30228 according to the TTC concept

Crop(group)	RP 30228 input values	Remarks
Plant	10% of iprodione parent STMR	derived from residues results as shown in CA 6.3.3
Bovine fat	0.0002	median residue for RP30228 (expected median calculated from feeding study prorated acc. to %TRR in metabolism)
Bovine liver	0.0001	
Bovine kidney	0.0003	
Poultry fat	0.0001	
Poultry liver	0.0005	
Milk	0.0001	
Exposure	Max: 218% of 0.0015 mg/kg bw/d = **3.3 µg/kg bw/d**	

RP 32490

Metabolite RP 32490 is found in most plants. As shown in CA 6.3.3 the compound can be realistically estimated with 10% of parent. In most animal matrices is identified as main metabolite. These values lead to an exposure of 3.3 µg/kg bw/d which is above the trigger of 1.5 µg/kg bw/d.

Also in the rat metabolism, RP 32490 was found to be major metabolite. Therefore, its toxicological properties can be regarded as covered by parent Iprodione.

For that reason, the compound is included into the risk assessment for iprodione by 10%.

Table 6.9-7: Residue values used for the exposure assessment of RP 32490 according to the TTC concept

Crop(group)	RP 32490 input values	Remarks
Plant	10% of iprodione parent STMR	derived from residues results as shown in CA 6.3.3
Swine muscle	0.0001	median residue for RP32490 (expected median calculated from feeding study prorated acc. to %TRR in metabolism)
Swine fat	0.0002	
Swine liver	0.0003	
Swine kidney	0.0004	
Bovine muscle	0.0016	
Bovine fat	0.0038	
Bovine liver	0.0055	
Bovine kidney	0.0066	
Poultry fat	0.0019	
Poultry liver	0.0028	
Milk	0.0005	
Poultry egg	0.0008	
Exposure	Max: 218% of 0.0015 mg/kg bw/d = **3.3 µg/kg bw/d**	

3,5-Dichloroaniline

3,5-Dichloroaniline (3,5-DCA, RP 36596) was regarded as compound of special concern because in the past no toxicological data were available and therefore extrapolations to other chloroanilines known as critical were done.

As iprodione metabolite, 3,5-DCA was identified in relatively high amounts in the hydrolysis studies investigating the behavior during processing (CA 6.5.1). In processing studies simulating crop processing (CA 6.5.2) it was found in lower amounts compared to the hydrolysis in solvent.

In order to quantify the levels in processed plant products basing on concentration of parent iprodione in raw agricultural commodities factors were derived (as explained in CA 6.5.3)

Transfer Factor 2
(to calculate concentration of 3,5-dichloroaniline in PF from concentration of iprodione in RAC):

Cooked commodity	**0.05**
Juice	**0.02**
Canned commodity	**0.15**

For a general exposure calculation of 3,5-DCA these factors were used for all crops which are used in cooking and sterilizing processes and juice production.

In addition to these findings, 3,5-DCA was separately analysed in all supervised field studies conducted in the last seasons. It turned out that the compound was only found in RAC very rarely and in very low amounts slightly above the LOQ of 0.01 mg/kg. These quantifiable residues were detected in lettuce and Lamb´s lettuce which are crops with a relatively high level of iprodione residues (MRL proposal 30 mg/kg). In other crops with generally lower residue level, 3,5-DCA was not detected in edible matrices.

Combining all these results leads to the following conclusion: Despite the fact that 3,5-DCA was never identified as plant metabolite, it can be found very rarely in crops with high residue levels. However, the identification of a conjugate proved that 3,5-DCA must have been present. Due to its high tendency to form conjugates, it is very unlikely to be found in higher amounts.

As consequence of these findings, an exposure calculation was performed with input values as shown in the following table. Due to the special concern of the compound, a worst case assumption using MRL values was made. The calculations were done using the VELS model (version 2.0) which is suitable for compounds mainly occurring in processed commodities.

Table 6.9-8: Input values used for the chronic exposure assessment of RP 32596 (3,5-DCA)

Crop(group)	RP 36596 input values	Remarks
Lemons	0.12	MRL x 0.02 (juice)
Almonds	0.001	MRL x 0.05 (cooked)
Hazelnuts	0.015	MRL x 0.05 (cooked)
Apples	0.35	MRL x 0.05 (cooked)
	0.14	MRL x 0.02 (juice)
Peaches	0.75	MRL x 0.15 (canned)
	0.1	MRL x 0.02 (juice)
Plums	0.225	MRL x 0.15 (canned)
	0.03	MRL x 0.02 (juice)
Table and wine grapes	0.3	MRL x 0.02 (juice)
Strawberries	2.25	MRL x 0.15 (canned)
	0.3	MRL x 0.02 (juice)
Blackberries	2.25	MRL x 0.15 (canned)
	0.3	MRL x 0.02 (juice)
Raspberries	2.25	MRL x 0.15 (canned)
	0.3	MRL x 0.02 (juice)
Currants (red, black, white)	2.25	MRL x 0.15 (canned)
	0.3	MRL x 0.02 (juice)
Carrots	0.04	MRL x 0.05 (cooked)
	0.016	MRL x 0.02 (juice)
Onions	0.03	MRL x 0.15 (canned)
Tomatoes	0.6	MRL x 0.15 (canned)
	0.08	MRL x 0.02 (juice)
Aubergines (egg plants)	0.2	MRL x 0.05 (cooked)
Peppers	0.4	MRL x 0.05 (cooked)
Gherkins	0.45	MRL x 0.15 (canned)
Courgettes	0.15	MRL x 0.05 (cooked)
Broccoli	0.02	MRL x 0.05 (cooked)
Cauliflower	0.02	MRL x 0.05 (cooked)
Brussels sprouts	0.035	MRL x 0.05 (cooked)
Lettuce	0.03	HR, residue trials
Lamb´s lettuce	0.03	HR, residue trials
Witloof	0.15	MRL x 0.05 (cooked)
Herbs	1.5	MRL x 0.05 (cooked)
Beans (fresh, with pods)	0.05	MRL x 0.05 (cooked)
Beans (fresh, without	0.15	MRL x 0.15 (canned)
Peas (fresh, w/o pods)	0.015	MRL x 0.05 (cooked)
Asparagus	0.001	MRL x 0.05 (cooked)
Lentils (dry)	0.01	MRL x 0.05 (cooked)
Peas (dry)	0.01	MRL x 0.05 (cooked)
Rice grain	0.15	MRL x 0.05 (cooked)
Exposure	Max: 117% of 0.0015 mg/kg bw/d = **1.75 µg/kg bw/d** Max: 14% of 0.0125 mg/kg bw/d = **1.75 µg/kg bw/d**	

The exposure calculation based on MRL values and conversion factors derived from the more critical processing study led to exceedance of the trigger value of 1.5 µg/kg bw/d. Due to the special concern of the compound a 28 day-study was conducted to derive an ADI value. The exposure as calculated is well covered by this ADI of 12.5 µg/kg bw/d.

Based on these results it is not regarded necessary to include 3,5-dichloroaniline (RP32596) into the residue definition.

As described in the toxicological evaluation of 3,5-dichloroaniline (CA 5.8.1) reversible methemoglobin formation was observed after acute intra peritoneal exposure. Therefore, an acute risk assessment was performed in addition using the ADI of 0.0125 mg/kg bw/d as acute reference dose. For this purpose, the acute VELS-model (version 2.0) was used with input values as defined in the model for the respective crops (cases 1 and 2a/2b: HR, case 3: STMR):

Table 6.9-9: Input values used for the acute exposure assessment of RP 32596 (3,5-DCA)

Crop(group)	RP 36596 input values	Remarks
Lemons	0.07	STMR x 0.02 (juice)
Apples / pears	0.09	STMR x 0.05 (cooked)
	0.036	STMR x 0.02 (juice)
Peaches	0.218	STMR x 0.15 (canned)
	0.029	STMR x 0.02 (juice)
Plums	0.035	STMR x 0.15 (canned)
	0.005	STMR x 0.02 (juice)
Table and wine grapes	0.5	HR x 0.05 (cooked)
	0.076	STMR x 0.02 (juice)
Strawberries	0.33	STMR x 0.15 (canned)
	0.044	STMR x 0.02 (juice)
Blackberries	0.428	STMR x 0.15 (canned)
	0.057	STMR x 0.02 (juice)
Raspberries	0.428	STMR x 0.15 (canned)
	0.057	STMR x 0.02 (juice)
Currants (red, black, white)	0.48	STMR x 0.15 (canned)
	0.064	STMR x 0.02 (juice)
Carrots	0.023	STMR x 0.05 (cooked)
	0.003	STMR x 0.02 (juice)
Onions	0.017	STMR x 0.15 (canned)
Tomatoes	0.245	STMR x 0.15 (canned)
	0.024	STMR x 0.02 (juice)
Aubergines (egg plants)	0.08	STMR x 0.05 (cooked)
Peppers	0.2	STMR x 0.05 (cooked)
Cucumber	0.68	STMR x 0.15 (canned)
Courgettes	0.023	STMR x 0.05 (cooked)
Broccoli	0.017	STMR x 0.05 (cooked)
Cauliflower	0.017	STMR x 0.05 (cooked)
Brussels sprouts	0.012	STMR x 0.05 (cooked)
Lettuce	0.03	HR
Lamb´s lettuce	0.03	HR
Beans (fresh, with pods)	0.028	STMR x 0.05 (cooked)
Peas (fresh, w/o pods)	0.008	STMR x 0.05 (cooked)
Lentils (dry)	0.01	STMR x 0.05 (cooked)
Peas (dry)	0.01	STMR x 0.05 (cooked)
Rice grain	0.15	STMR x 0.05 (cooked)

The estimate performed results in utilisations of the reference dose of 20% each for grape juice and canned peaches and of 15% for apple juice. The contribution of the processed commodities from other target crops is considerably lower.

RP 36112

Table 6.9-10: Residue values used for the exposure assessment of RP 36112 according to the TTC concept

Crop(group)	RP 36112 input values	Remarks
Carrot	0.032	20% of parent STMR [1)]
Rice	0.083	10% of parent STMR [1)]
Other plants	1% of iprodione parent STMR	worst case assumption
Bovine muscle	0.0001	median residue for RP36112 (expected median calculated from feeding study prorated acc. to %TRR in metabolism)
Bovine fat	0.0001	
Bovine liver	0.0002	
Bovine kidney	0.0009	
Poultry liver	0.0002	
milk	0.0001	
Poultry egg	0.0004	
Exposure	Max: 25% of 0.0015 mg/kg bw/d = **0.38 µg/kg bw/d**	

1) Based on metabolism study

RP 25040

Table 6.9-11: Residue values used for the exposure assessment of RP 25040 according to the TTC concept

Crop(group)	RP 25040 input values	Remarks
Rice	0.08	10% of parent STMR
Other plants	1% of iprodione parent STMR	worst case assumption
Exposure	Max: 23% of 0.0015 mg/kg bw/d = **0.35 µg/kg bw/d**	

RP 36221

Residue values used for the exposure assessment of RP 36221 according to the TTC concept

Crop(group)	RP 36221 input values	Remarks
Carrot	0.032	20% of parent STMR [1)]
Rice	0.08	10% of parent STMR [1)]
Bovine liver	0.0001	median residue for RP36221 (expected median calculated from feeding study prorated acc. to %TRR in metabolism)
Bovine kidney	0.0011	
Poultry liver	0.0001	
Exposure	Max: 25% of 0.0015 mg/kg bw/d = **0.38 µg/kg bw/d**	

RP 36115

Table 6.9-12: Residue values used for the exposure assessment of RP 36115 according to the TTC concept

Crop(group)	RP 36115 input values	Remarks
Carrot	0.048	25% of parent STMR
Swine kidney	0.002	median residue for RP36115 (expected median calculated from feeding study prorated acc. to %TRR in metabolism)
Bovine muscle	0.0001	
Bovine fat	0.0001	
Bovine liver	0.0008	
Bovine kidney	0.0042	
Poultry liver	0.0004	
Milk	0.0001	
Poultry egg	0.0003	
Exposure	Max: 27% of 0.0015 mg/kg bw/d = **0.41 µg/kg bw/d**	

RP36114

Table 6.9-13: Residue values used for the exposure assessment of RP 36114 according to the TTC concept

Crop(group)	RP 36114 input values	Remarks
Swine kidney	0.0001	median residue for RP36114 (expected median calculated from feeding study prorated acc. to %TRR in metabolism)
Bovine liver	0.0004	
Bovine kidney	0.0020	
Poultry liver	0.0002	
Milk	0.0020	
Exposure	Max: 5% of 0.0015 mg/kg bw/d = **0.08 µg/kg bw/d**	

RP 44247

Table 6.9-14: Residue values used for the exposure assessment of RP 44247 according to the TTC concept

Crop(group)	RP 44247 input values	Remarks
Carrot	0.04	25% of parent STMR
Swine liver	0.0001	median residue for RP44247 (expected median calculated from feeding study prorated acc. to %TRR in metabolism)
Bovine fat	0.0001	
Bovine liver	0.0016	
Poultry liver	0.0034	
Poultry egg	0.0002	
Exposure	Max: 8% of 0.0015 mg/kg bw/d = **0.12 µg/kg bw/d**	

DCHPU

Table 6.9-15: Residue values used for the exposure assessment of DCPHU according to the TTC concept

Crop(group)	DCPHU input values	Remarks
Swine liver	0.0001	median residue for DCHPU (expected median calculated from feeding study prorated acc. to %TRR in metabolism)
Swine kidney	0.0007	
Bovine muscle	0.0002	
Bovine fat	0.0001	
Bovine liver	0.0025	
Bovine kidney	0.0127	
Exposure	Max: 0.1% of 0.0015 mg/kg bw/d = **0.002 µg/kg bw/d**	

M610F007

M610F007 is the conjugate of 3,5-dichloroaniline and naturally occurring glutamic acid which was newly identified in the carrot metabolism study (CA 6.2). Also in field trials, it was found exclusively and in relatively high amounts in carrot roots.

Table 6.9-16: Residue values used for the exposure assessment of M610F007 according to the TTC concept

Crop(group)	M610F007 input values	Remarks
carrot	0.38	HR
Lamb´s lettuce	0.08	HR
Exposure	Max: 67% of 0.0015 mg/kg bw/d = **1 µg/kg bw/d**	

RP 37176

Metabolite RP 37176 was formed during hydrolysis study (CA 6.5.1) up to 20%. In the carrot processing study (CA 6.5.2), however, it was only found at the LOQ in the sterilised product. Based on this result a "transfer factor 2" calculating the concentration of metabolite in the processed commodity from the amount of parent in the RAC was derived which is 0.02. This factor was multiplied with the MRLs of all potentially sterilised plant matrices to estimate a chronic exposure using the VELS model.

Table 6.9-17: Input values used for the exposure assessment of RP 37176

Crop(group)	RP 37176 input values	Remarks
Apples	0.14	MRL x 0.02 (canned)
Pear	0.14	MRL x 0.02 (canned)
Peaches	0.10	MRL x 0.02 (canned)
Plums	0.03	MRL x 0.02 (canned)
Strawberries	0.30	MRL x 0.02 (canned)
Blackberries	0.30	MRL x 0.02 (canned)
Raspberries	0.30	MRL x 0.02 (canned)
Currants (red, black, white)	0.30	MRL x 0.02 (canned)
Carrots	0.02	MRL x 0.02 (canned)
Onions	0.004	MRL x 0.02 (canned)
Garlic	0.004	MRL x 0.02 (canned)
Tomatoes	0.08	MRL x 0.02 (canned)
Aubergines (egg plants)	0.08	MRL x 0.02 (canned)
Peppers	0.16	MRL x 0.02 (canned)
Gherkins	0.06	MRL x 0.02 (canned)
Courgettes	0.06	MRL x 0.02 (canned)
Broccoli	0.008	MRL x 0.02 (canned)
Cauliflower	0.008	MRL x 0.02 (canned)
Lettuce	0.03	HR, residue trials
Lamb´s lettuce	0.03	HR, residue trials
Witloof	0.15	MRL x 0.02 (canned)
Herbs	0.03	HR, residue trials
Peas (fresh, w/o pods)	0.01	MRL x 0.02 (canned)
Asparagus	0.004	MRL x 0.02 (canned)
Lentils (dry)	0.004	MRL x 0.02 (canned)
Exposure	Max: 13.3% of 0.0015 mg/kg bw/d = **0.199 µg/kg bw/d**	

For its constitutional isomer of RP 36233 which was only found in traces in the hydrolysis study (R014571, already peer reviewed) the exposure cannot be quantified. It can be regarded as <0.0025 µg/kg bw/d.

Risk assessments for the metabolites according to the TTC Concept

According to the TTC (Threshold of Toxicological Concern) concept for metabolites the chronic risk assessments for the twelve iprodione metabolites were performed.

The metabolites **RP 30228** and **RP 32490** exceeded the exposure threshold value of 1.5 µg/kg bw/d for Cramer Class III compounds.

Metabolite RP 30228 was identified as minor component in rat and its toxicity turned out to be lower than parent iprodione. RP 32490 is the predominant metabolite in rat metabolism.

Based on values obtained from separate analysis both compound were included into the residue definition for risk assessment and accounted for 10% of parent each.

Also the exposure of the degradation product **3,5-dichloroaniline** is expected to be above 1.5 µg/kg bw/d. For this compound a separate ADI was derived which covers the exposure sufficiently in a chronic as well as acute assessment.

The calculated exposure values for the compounds **RP 36112**, **RP25040**, **RP36221**, **RP36115**, **RP36114**, **RP44247**, **M610F007** and **RP37176** turned out to be below 1.5 µg/kg bw/d. In case of one compound, **DCHPU**, an exposure below 0.0025 µg/kg bw/d was obtained.

The metabolites were grouped based on structural similarity and metabolic pathway concordance and representatives were further tested. Details of the exposure calculations are shown on the following pages. Grouping and testing is explained and reported in CA 5.8.

Based on these data, a long-term intake of residues of these metabolites is unlikely to present a public health concern and they can be assumed to be not relevant for consumer safety.

Table 6.9-18: TMDI calculation for RP30228 with PRIMo Model (rev 2.0)

RP30228			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0.0015	ARfD (mg/kg bw):	
Source of ADI:	TTC	Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Prepare workbook for refined calculations

Undo refined calculations

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	
23	218
No of diets exceeding ADI:	5

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)
217.6	DE child	144.8	Apples	32.0	Table and wine grapes	7.4	Pears	
126.1	NL child	76.0	Apples	19.2	Table and wine grapes	5.1	Pears	
124.1	WHO Cluster diet B	54.0	Table and wine grapes	20.6	Tomatoes	12.1	Apples	
122.2	FR all population	103.6	Table and wine grapes	5.7	Apples	2.9	Tomatoes	
105.9	PT General population	69.8	Table and wine grapes	12.6	Apples	6.0	Tomatoes	
81.5	IE adult	29.4	Table and wine grapes	9.9	Apples	8.0	Pears	
72.0	WHO cluster diet E	45.2	Table and wine grapes	10.2	Apples	3.5	Tomatoes	
63.0	FR toddler	31.5	Apples	9.1	Strawberries	5.2	Table and wine grapes	
58.0	DK child	27.9	Apples	8.1	Pears	4.9	Cucumbers	
57.9	DK adult	37.1	Table and wine grapes	9.4	Apples	2.8	Tomatoes	
52.4	FR infant	30.0	Apples	7.2	Strawberries	3.8	Pears	
49.5	NL general	21.7	Table and wine grapes	14.2	Apples	2.8	Tomatoes	
47.9	UK Toddler	20.5	Apples	7.1	Table and wine grapes	3.9	Tomatoes	
47.8	PL general population	24.5	Apples	8.1	Table and wine grapes	5.9	Tomatoes	
45.2	SE general population 90th percentile	12.6	Apples	5.7	Table and wine grapes	5.1	Tomatoes	
42.5	UK Adult	28.6	Table and wine grapes	4.9	Apples	2.9	Tomatoes	
42.3	WHO cluster diet D	13.9	Table and wine grapes	8.0	Apples	6.7	Tomatoes	
42.1	UK vegetarian	22.5	Table and wine grapes	7.1	Apples	4.1	Tomatoes	
41.1	WHO Cluster diet F	18.5	Table and wine grapes	7.9	Apples	4.5	Tomatoes	
39.6	WHO regional European diet	9.7	Table and wine grapes	8.0	Apples	7.3	Tomatoes	
39.1	ES adult	11.6	Table and wine grapes	9.2	Apples	5.2	Tomatoes	
37.8	UK Infant	18.8	Apples	3.5	Rice	3.2	Strawberries	
37.6	IT kids/toddler	10.6	Apples	9.5	Tomatoes	4.2	Pears	
36.8	ES child	13.7	Apples	6.5	Tomatoes	5.3	Pears	
33.7	IT adult	9.5	Apples	7.8	Tomatoes	3.6	Peaches	
33.5	LT adult	22.4	Apples	4.1	Tomatoes	1.9	Pears	
22.6	FI adult	8.2	Table and wine grapes	4.8	Apples	2.8	Tomatoes	

Conclusion:

The estimated Theoretical Maximum Daily Intakes based on MS and WHO diets and pTMRLs were in the range of 22.6 % to 218 % of the ADI.
For 5 diets the ADI is exceeded. Further refinements of the dietary intake estimates have not been performed. A public health risk can not be excluded at the moment.

Table 6.9-19: TMDI calculation for RP32490 with PRIMo Model (rev 2.0)

RP32490			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0.0015	ARfD (mg/kg bw):	
Source of ADI:	TTC	Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Prepare workbook for refined calculations

Undo refined calculations

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	
23	218
No of diets exceeding ADI:	5

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)
217.8	DE child	144.8	Apples	32.0	Table and wine grapes	7.4	Pears	
126.6	NL child	76.0	Apples	19.2	Table and wine grapes	5.1	Pears	
123.0	WHO Cluster diet B	54.0	Table and wine grapes	20.6	Tomatoes	12.1	Apples	
121.9	FR all population	103.6	Table and wine grapes	5.7	Apples	2.9	Tomatoes	
105.8	PT General population	69.8	Table and wine grapes	12.6	Apples	6.0	Tomatoes	
79.7	IE adult	29.4	Table and wine grapes	9.9	Apples	8.0	Pears	
71.8	WHO cluster diet E	45.2	Table and wine grapes	10.2	Apples	3.5	Tomatoes	
63.8	FR toddler	31.5	Apples	9.1	Strawberries	5.2	Table and wine grapes	
58.1	DK child	27.9	Apples	8.1	Pears	4.9	Cucumbers	
58.0	DK adult	37.1	Table and wine grapes	9.4	Apples	2.8	Tomatoes	
52.9	FR infant	30.0	Apples	7.2	Strawberries	3.8	Pears	
49.5	NL general	21.7	Table and wine grapes	14.2	Apples	2.8	Tomatoes	
48.4	UK Toddler	20.5	Apples	7.1	Table and wine grapes	3.9	Tomatoes	
47.3	PL general population	24.5	Apples	8.1	Table and wine grapes	5.9	Tomatoes	
45.2	SE general population 90th percentile	12.6	Apples	5.7	Table and wine grapes	5.1	Tomatoes	
42.5	UK Adult	28.6	Table and wine grapes	4.9	Apples	2.9	Tomatoes	
42.1	UK vegetarian	22.5	Table and wine grapes	7.1	Apples	4.1	Tomatoes	
42.1	WHO cluster diet D	13.9	Table and wine grapes	8.0	Apples	6.7	Tomatoes	
40.7	WHO Cluster diet F	18.5	Table and wine grapes	7.9	Apples	4.5	Tomatoes	
39.5	WHO regional European diet	9.7	Table and wine grapes	8.0	Apples	7.3	Tomatoes	
39.3	ES adult	11.6	Table and wine grapes	9.2	Apples	5.2	Tomatoes	
38.7	UK infant	18.8	Apples	3.5	Rice	3.2	Strawberries	
37.3	IT kids/toddler	10.6	Apples	9.5	Tomatoes	4.2	Pears	
37.2	ES child	13.7	Apples	6.5	Tomatoes	5.3	Pears	
33.5	LT adult	22.4	Apples	4.1	Tomatoes	1.9	Pears	
33.3	IT adult	9.5	Apples	7.8	Tomatoes	3.6	Peaches	
22.6	FI adult	8.2	Table and wine grapes	4.8	Apples	2.8	Tomatoes	

Conclusion:

The estimated Theoretical Maximum Daily Intakes based on MS and WHO diets and pTMRLs were in the range of 22.6 % to 218 % of the ADI.
For 5 diets the ADI is exceeded. Further refinements of the dietary intake estimates have not been performed. A public health risk can not be excluded at the moment.

Table 6.9-20: TMDI calculation for RP32596 with VELS Model

Active substance: DCA	Total intake in mg/kg bw: 0.001752006
ADI (mg/kg bw): 0.0125	% ADI 14.01604954
Mean bodyweight (kg): 16.15	Calculation method: TMDI

Code number	Commodity	chronic			
		Mean consumption (g/d)	MRL (mg/kg)	STMR (mg/kg)	Intake (mg/kg bw)
0231010	Tomatoes, processed	10.3	0.6		0.000382663
0130010	Apples, portion in juice	150.2	0.036		0.000334811
0151010	Grapes, portion in juice	12	0.3		0.00022291
0130010	Apples, processed	6.8	0.35		0.000147368
0152000	Strawberries, processed	1	2.25		0.000139319
0231020	Peppers, processed	1.5	1.2		0.000111455
0140030	Peaches and nectarines, processed	1.4	0.75		6.50155E-05
0153030	Raspberries, processed	0.4	2.25		5.57276E-05
0154010	Blueberries, processed	0.2	2.25		2.78638E-05
0154030	Currants, processed	0.2	2.25		2.78638E-05
0500060	Rice, processed	2.7	0.15		2.50774E-05
0256040	Parsley, processed	0.2	1.5		1.85759E-05
0154030	Currants, portion in juice	1	0.3		1.85759E-05
0213020	Carrots, processed	6.9	0.035		1.49536E-05
0153010	Blackberries, processed	0.1	2.25		1.39319E-05
0256010	Chervil, processed*	0.1	1.5		9.28793E-06
0256020	Chives, processed*	0.1	1.5		9.28793E-06
0256030	Dill, processed*	0.1	1.5		9.28793E-06
0256060	Rosemary, processed*	0.1	1.5		9.28793E-06
0256070	Marjoram, portion in dried product*	0.1	1.5		9.28793E-06
0256070	Thyme, processed*	0.1	1.5		9.28793E-06
0256080	Basil, processed*	0.1	1.5		9.28793E-06
0130020	Pears, portion in juice	4	0.036		8.91641E-06
0140040	Plums, processed	0.6	0.225		8.35913E-06
0232030	Courgette, processed	0.9	0.15		8.35913E-06
0153030	Raspberries, portion in juice	0.4	0.3		7.43034E-06
0130020	Pears, processed	0.3	0.35		6.50155E-06
0213020	Carrots, portion in juice	5.9	0.016	0.0032	5.8452E-06
0220020	Onions, processed	2.7	0.03	0.001	5.01548E-06
0152000	Strawberries, portion in juice	0.2	0.3		3.71517E-06
0260010	Beans (pods and succulent immature seeds), processed	1.1	0.05		3.40557E-06
0251000	a) Lettuce and other salad plants including Brassicacea	1.4	0.03		2.60062E-06
0140030	Peaches, portion in juice	0.4	0.1		2.47678E-06
0241020	Cauliflower, processed	2.1	0.015		1.95046E-06
0153010	Blackberries, portion in juice*	0.1	0.3		1.85759E-06
0154010	Blueberries, portion in juice*	0.1	0.3		1.85759E-06
0251020	Other lettuce species, raw	1	0.03		1.85759E-06

0241010	Broccoli, processed	1.8	0.015		1.67183E-06
0231010	Tomatoes, portion in juice	0.3	0.08		1.48607E-06
0231030	Egg plant, processed	0.1	0.2		1.23839E-06
0120060	Hazelnuts	1.3	0.015		1.20743E-06
0242010	Brussels sprouts, processed	0.4	0.035		8.66873E-07
0140040	Plums, portion in juice	0.4	0.03		7.43034E-07
0251030	Endive, raw	0.2	0.03		3.71517E-07
0220010	Garlic, processed	0.1	0.03	0.001	1.85759E-07
0251010	Lamb´s lettuce, raw	0.1	0.03		1.85759E-07
0251020	Other lettuce species, processed*	0.1	0.03		1.85759E-07
0251040	Cress, total*	0.1	0.03		1.85759E-07
0260030	Peas, processed*	0.1	0.015		9.28793E-08
0300020	Lentils dry, processed	0.1	0.01		6.19195E-08
0300030	Peas, portion in dried product*	0.1	0.01		6.19195E-08
0120010	Almonds	0.5	0.001		3.09598E-08
0270010	Asparagus, processed	0.3	0.001		1.85759E-08

Table 6.9-21: Acute risk assessment for RP32596 with VELS Model

Code number	Commodity	acute								
		Percentile	Large portion (g)	Unit weight (g)	HR or HR-P (mg/kg)	STMR-P (mg/kg)	Variability-factor	Case	Intake (mg/kg bw)	ARfD (%)
151010	Grapes, portion in juice	97.5	531.3			0.076		3	0.002500235	20.0
140030	Peaches and nectarines, processed	97.5	181.8	136		0.218		3	0.002454019	19.6
130010	Apples, portion in juice	97.5	822.9			0.036		3	0.001834328	14.7
154030	Currants, processed	97.5	46.4			0.48		3	0.001379071	11.0
153030	Raspberries, processed	97.5	40.8			0.428		3	0.001081263	8.7
130010	Apples, processed	97.5	183	182		0.09		3	0.001019814	8.2
153030	Raspberries, portion in juice	95	193.6			0.057		3	0.000683294	5.5
154030	Currants, portion in juice	97.5	163.3			0.064		3	0.000647133	5.2
130020	Pears, portion in juice	97.5	282.9			0.036		3	0.000630613	5.0
130020	Pears, processed	95	98.7	207		0.09		3	0.000550031	4.4
140030	Peaches, portion in juice	90	289.2			0.029		3	0.000519307	4.2
152000	Strawberries, processed	97.5	25			0.33		3	0.000510836	4.1
232010	Cucumber, processed	97.5	106.3	458		0.068		3	0.000447579	3.6
231010	Tomatoes, portion in juice	MAX	281.6			0.024		3	0.000418477	3.3
153010	Blackberries, processed	97.5	15.6			0.428		3	0.000413424	3.3
140040	Plums, processed	97.5	184.1	79		0.035		3	0.000398978	3.2
152000	Strawberries, portion in juice	90	74.8			0.044		3	0.000203789	1.6
231010	Tomatoes, processed	97.5	111.2	99	0.245			2a/2b	0.000185077	1.5
260010	Beans (pods and succulent immature seeds), processed	97.5	106.6		0.028			1	0.000184817	1.5
110030	Lemons, portion in juice	97.5	38.5			0.07		3	0.000166873	1.3
500060	Rice, processed	97.5	56.9			0.045		3	0.000158545	1.3
232030	Courgette, processed	97.5	99.3	210		0.023		3	0.000141418	1.1
213020	Carrots, portion in juice	97.5	692.8			0.003		3	0.000128693	1.0
242010	Brussels sprouts, processed	90	122			0.012		3	9.06502E-05	0.7
251010	Lamb´s lettuce, raw	90	41.2		0.03			1	7.65325E-05	0.6
140040	Plums, portion in juice	95	225.2			0.005		3	6.97214E-05	0.6
213020	Carrots, processed	97.5	97.1	62	0.023			2a/2b	4.99876E-05	0.4
153010	Blackberries, portion in juice*	MAX	11.3			0.057		3	3.98824E-05	0.3
270010	Asparagus, processed	97.5	155.4	25	0.001			2a/2b	8.0743E-06	0.1
300020	Lentils dry, processed	90	26.9			0.003		3	4.9969E-06	0.0
260030	Peas, processed*	MAX	6.9		0.008			1	3.41796E-06	0.0

Table 6.9-22: TMDI calculation for RP36112 with PRIMo Model (rev 2.0)

RP36112			
Status of the active substance:		Code no.:	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0.0015	ARfD (mg/kg bw):	
Source of ADI:	TTC	Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Prepare workbook for refined calculations

Undo refined calculations

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
3 25

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)
25.3	DE child	14.5	Apples	3.2	Table and wine grapes	2.2	Carrots	
15.8	PT General population	7.0	Table and wine grapes	4.3	Rice	1.4	Carrots	
15.5	NL child	7.6	Apples	2.0	Rice	1.9	Table and wine grapes	
15.5	WHO Cluster diet B	5.4	Table and wine grapes	2.9	Rice	2.1	Tomatoes	
13.4	FR all population	10.4	Table and wine grapes	0.6	Carrots	0.6	Rice	
13.3	FR toddler	5.2	Carrots	3.1	Apples	2.0	Rice	
11.2	FR infant	5.6	Carrots	3.0	Apples	0.7	Strawberries	
9.8	UK Infant	3.5	Rice	2.8	Carrots	1.9	Apples	
9.5	IE adult	2.9	Table and wine grapes	1.0	Apples	0.9	Rice	
9.2	WHO cluster diet E	4.5	Table and wine grapes	1.2	Rice	1.0	Apples	
9.2	DK child	2.9	Carrots	2.8	Apples	0.8	Pears	
8.8	UK Toddler	3.2	Rice	2.0	Apples	1.1	Carrots	
8.3	SE general population 90th percentile	2.2	Rice	1.8	Carrots	1.3	Apples	
7.5	WHO cluster diet D	3.1	Rice	1.4	Table and wine grapes	0.8	Apples	
7.1	DK adult	3.7	Table and wine grapes	0.9	Carrots	0.9	Apples	
6.6	UK vegetarian	2.2	Table and wine grapes	2.1	Rice	0.7	Apples	
6.5	ES child	2.7	Rice	1.4	Apples	0.7	Tomatoes	
6.4	UK Adult	2.9	Table and wine grapes	2.0	Rice	0.5	Apples	
6.2	NL general	2.2	Table and wine grapes	1.4	Apples	0.9	Rice	
6.1	WHO Cluster diet F	1.8	Table and wine grapes	1.2	Rice	1.0	Carrots	
5.7	WHO regional European diet	1.1	Rice	1.0	Table and wine grapes	0.8	Apples	
5.5	ES adult	1.3	Rice	1.2	Table and wine grapes	0.9	Apples	
5.3	PL general population	2.5	Apples	0.8	Table and wine grapes	0.6	Carrots	
5.1	IT kids/toddler	1.1	Apples	1.1	Rice	1.0	Tomatoes	
4.8	LT adult	2.2	Apples	1.2	Rice	0.4	Tomatoes	
4.5	IT adult	1.0	Rice	1.0	Apples	0.8	Tomatoes	
3.2	FI adult	0.8	Table and wine grapes	0.6	Rice	0.5	Apples	

Conclusion:
The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of RP36112 is unlikely to present a public health concern.

Table 6.9-23: TMDI calculation for RP25040 with PRIMo Model (rev 2.0)

RP25040			
Status of the active substance:		Code no:	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0.0015	ARfD (mg/kg bw):	
Source of ADI:	TTC	Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Prepare workbook for refined calculations

Undo refined calculations

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
	3 23
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)
23.0	DE child	14.5	Apples	3.2	Table and wine grapes	1.5	Rice	
14.9	WHO Cluster diet B	5.4	Table and wine grapes	2.9	Rice	2.1	Tomatoes	
14.5	PT General population	7.0	Table and wine grapes	4.3	Rice	1.3	Apples	
14.3	NL child	7.6	Apples	2.0	Rice	1.9	Table and wine grapes	
12.7	FR all population	10.4	Table and wine grapes	0.6	Rice	0.6	Apples	
8.8	IE adult	2.9	Table and wine grapes	1.0	Apples	0.9	Rice	
8.2	WHO cluster diet E	4.5	Table and wine grapes	1.2	Rice	1.0	Apples	
8.0	FR toddler	3.1	Apples	2.0	Rice	0.9	Strawberries	
7.6	UK Toddler	3.2	Rice	2.0	Apples	0.7	Table and wine grapes	
6.9	WHO cluster diet D	3.1	Rice	1.4	Table and wine grapes	0.8	Apples	
6.9	UK Infant	3.5	Rice	1.9	Apples	0.3	Strawberries	
6.5	SE general population 90th percentile	2.2	Rice	1.3	Apples	0.6	Table and wine grapes	
6.3	DK child	2.8	Apples	0.8	Pears	0.6	Rice	
6.2	DK adult	3.7	Table and wine grapes	0.9	Apples	0.5	Rice	
6.1	UK vegetarian	2.2	Table and wine grapes	2.1	Rice	0.7	Apples	
6.1	ES child	2.7	Rice	1.4	Apples	0.7	Tomatoes	
6.1	UK Adult	2.9	Table and wine grapes	2.0	Rice	0.5	Apples	
5.7	NL general	2.2	Table and wine grapes	1.4	Apples	0.9	Rice	
5.6	FR Infant	3.0	Apples	0.7	Strawberries	0.5	Rice	
5.1	ES adult	1.3	Rice	1.2	Table and wine grapes	0.9	Apples	
5.1	WHO Cluster diet F	1.8	Table and wine grapes	1.2	Rice	0.8	Apples	
4.9	WHO regional European diet	1.1	Rice	1.0	Table and wine grapes	0.8	Apples	
4.7	PL general population	2.5	Apples	0.8	Table and wine grapes	0.6	Tomatoes	
4.7	IT kids/toodler	1.1	Apples	1.1	Rice	1.0	Tomatoes	
4.4	LT adult	2.2	Apples	1.2	Rice	0.4	Tomatoes	
4.2	IT adult	1.0	Rice	1.0	Apples	0.8	Tomatoes	
2.8	FI adult	0.8	Table and wine grapes	0.6	Rice	0.5	Apples	

Conclusion:
The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of RP25040 is unlikely to present a public health concern.

Table 6.9-24: TMDI calculation for RP36221 with PRIMo Model (rev 2.0)

RP36221

Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0.0015	ARfD (mg/kg bw):	
Source of ADI:	TTC	Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Prepare workbook for refined calculations

Undo refined calculations

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
3 25

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)
25.0	DE child	14.5	Apples	3.2	Table and wine grapes	2.1	Carrots	
15.8	PT General population	7.0	Table and wine grapes	4.3	Rice	1.3	Carrots	
15.4	WHO Cluster diet B	5.4	Table and wine grapes	2.9	Rice	2.1	Tomatoes	
15.3	NL child	7.6	Apples	2.0	Rice	1.9	Table and wine grapes	
13.3	FR all population	10.4	Table and wine grapes	0.6	Rice	0.6	Carrots	
12.7	FR toddler	4.9	Carrots	3.1	Apples	2.0	Rice	
10.7	FR infant	5.3	Carrots	3.0	Apples	0.7	Strawberries	
9.4	IE adult	2.9	Table and wine grapes	1.0	Apples	0.9	Rice	
9.4	UK infant	3.5	Rice	2.6	Carrots	1.9	Apples	
9.1	WHO cluster diet E	4.5	Table and wine grapes	1.2	Rice	1.0	Apples	
8.9	DK child	2.8	Apples	2.7	Carrots	0.8	Pears	
8.6	UK Toddler	3.2	Rice	2.0	Apples	1.0	Carrots	
8.1	SE general population 90th percentile	2.2	Rice	1.7	Carrots	1.3	Apples	
7.4	WHO cluster diet D	3.1	Rice	1.4	Table and wine grapes	0.8	Apples	
7.0	DK adult	3.7	Table and wine grapes	0.9	Apples	0.9	Carrots	
6.5	UK vegetarian	2.2	Table and wine grapes	2.1	Rice	0.7	Apples	
6.4	ES child	2.7	Rice	1.4	Apples	0.7	Tomatoes	
6.4	UK Adult	2.9	Table and wine grapes	2.0	Rice	0.5	Apples	
6.1	NL general	2.2	Table and wine grapes	1.4	Apples	0.9	Rice	
6.0	WHO Cluster diet F	1.8	Table and wine grapes	1.2	Rice	0.9	Carrots	
5.6	WHO regional European diet	1.1	Rice	1.0	Table and wine grapes	0.8	Apples	
5.4	ES adult	1.3	Rice	1.2	Table and wine grapes	0.9	Apples	
5.3	PL general population	2.5	Apples	0.8	Table and wine grapes	0.6	Carrots	
5.0	IT kids/toddler	1.1	Apples	1.1	Rice	1.0	Tomatoes	
4.7	LT adult	2.2	Apples	1.2	Rice	0.4	Tomatoes	
4.5	IT adult	1.0	Rice	1.0	Apples	0.8	Tomatoes	
3.1	FI adult	0.8	Table and wine grapes	0.6	Rice	0.5	Apples	

Conclusion:
The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of RP36221 is unlikely to present a public health concern.

Table 6.9-25: TMDI calculation for RP36115 with PRIMo Model (rev 2.0)

RP36115			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0.0015	ARfD (mg/kg bw):	
Source of ADI:	TTC	Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Prepare workbook for refined calculations

Undo refined calculations

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	
4	27
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)
26.7	DE child	14.5	Apples	3.3	Carrots	3.2	Table and wine grapes	
18.5	WHO Cluster diet B	5.4	Table and wine grapes	2.9	Rice	2.2	Beetroot	
17.5	FR toddler	7.8	Carrots	3.1	Apples	2.0	Rice	
16.8	NL child	7.6	Apples	2.0	Rice	1.9	Table and wine grapes	
16.6	PT General population	7.0	Table and wine grapes	4.3	Rice	2.1	Carrots	
15.2	FR infant	8.5	Carrots	3.0	Apples	0.8	Turnips	
14.2	IE adult	2.9	Table and wine grapes	2.0	Parsnips	1.0	Carrots	
14.1	FR all population	10.4	Table and wine grapes	1.0	Carrots	0.6	Rice	
11.8	UK Infant	4.2	Carrots	3.5	Rice	1.9	Apples	
10.8	DK child	4.4	Carrots	2.8	Apples	0.8	Pears	
10.6	SE general population 90th percentile	2.7	Carrots	2.2	Rice	1.3	Apples	
10.1	WHO cluster diet E	4.5	Table and wine grapes	1.4	Carrots	1.2	Rice	
9.7	UK Toddler	3.2	Rice	2.0	Apples	1.7	Carrots	
8.3	WHO cluster diet D	3.1	Rice	1.4	Table and wine grapes	0.8	Apples	
8.1	WHO Cluster diet F	1.8	Table and wine grapes	1.5	Carrots	1.2	Rice	
7.7	DK adult	3.7	Table and wine grapes	1.4	Carrots	0.9	Apples	
7.0	UK vegetarian	2.2	Table and wine grapes	2.1	Rice	0.7	Carrots	
6.8	UK Adult	2.9	Table and wine grapes	2.0	Rice	0.6	Carrots	
6.8	NL general	2.2	Table and wine grapes	1.4	Apples	0.9	Rice	
6.8	ES child	2.7	Rice	1.4	Apples	0.7	Tomatoes	
6.7	PL general population	2.5	Apples	1.0	Carrots	0.8	Table and wine grapes	
6.6	WHO regional European diet	1.2	Carrots	1.1	Rice	1.0	Table and wine grapes	
5.6	ES adult	1.3	Rice	1.2	Table and wine grapes	0.9	Apples	
5.4	LT adult	2.2	Apples	1.2	Rice	0.6	Carrots	
5.3	IT kids/toddler	1.1	Apples	1.1	Rice	1.0	Tomatoes	
4.8	IT adult	1.0	Rice	1.0	Apples	0.8	Tomatoes	
3.7	FI adult	0.8	Table and wine grapes	0.6	Carrots	0.6	Rice	

Conclusion:

The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of RP36115 is unlikely to present a public health concern.

Table 6.9-26: TMDI calculation for RP36114 with PRIMo Model (rev 2.0)

36114			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0.0015	ARfD (mg/kg bw):	
Source of ADI:	TTC	Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Prepare workbook for refined calculations

Undo refined calculations

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	5
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)
5.3	FR toddler	5.3	Milk and cream,		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
5.2	UK Infant	5.2	Milk and cream,	0.0	Bovine: Kidney	0.0	Bovine: Liver	
3.9	NL child	3.9	Milk and cream,	0.0	Bovine: Liver	0.0	Bovine: Kidney	
3.4	FR infant	3.4	Milk and cream,		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
2.8	UK Toddler	2.8	Milk and cream,	0.0	Bovine: Kidney	0.0	Bovine: Liver	
1.9	DE child	1.9	Milk and cream,		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
1.7	DK child	1.7	Milk and cream,	0.0	Bovine: Liver		FRUIT (FRESH OR FROZEN)	
1.7	ES child	1.7	Milk and cream,	0.0	Bovine: Liver	0.0	Swine: Kidney	
1.7	SE general population 90th percentile	1.7	Milk and cream,		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
0.9	NL general	0.9	Milk and cream,	0.0	Bovine: Liver	0.0	Poultry: Liver	
0.8	FI adult	0.8	Milk and cream,		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
0.7	DK adult	0.7	Milk and cream,	0.0	Bovine: Liver		FRUIT (FRESH OR FROZEN)	
0.7	WHO cluster diet D	0.7	Milk and cream,	0.0	Bovine: Kidney	0.0	Bovine: Liver	
0.7	ES adult	0.7	Milk and cream,	0.0	Bovine: Liver	0.0	Swine: Kidney	
0.6	WHO regional European diet	0.6	Milk and cream,	0.0	Bovine: Kidney	0.0	Bovine: Liver	
0.5	WHO Cluster diet F	0.5	Milk and cream,	0.0	Bovine: Kidney	0.0	Bovine: Liver	
0.5	LT adult	0.5	Milk and cream,	0.0	Bovine: Liver		FRUIT (FRESH OR FROZEN)	
0.4	WHO Cluster diet B	0.4	Milk and cream,	0.0	Bovine: Kidney	0.0	Bovine: Liver	
0.4	UK vegetarian	0.4	Milk and cream,		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
0.4	UK Adult	0.4	Milk and cream,	0.0	Bovine: Liver	0.0	Bovine: Liver	
0.4	WHO cluster diet E	0.4	Milk and cream,	0.0	Bovine: Liver		FRUIT (FRESH OR FROZEN)	
0.4	IE adult	0.4	Milk and cream,		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
0.4	FR all population	0.4	Milk and cream,		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	IT adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	IT adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	IT adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	IT 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 36114 is unlikely to present a public health concern.

Table 6.9-27: TMDI calculation for RP44247 with PRIMo Model (rev 2.0)

RP44247			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0.0015	ARfD (mg/kg bw):	
Source of ADI:	TTC	Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Prepare workbook for refined calculations

Undo refined calculations

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
	0 8
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)
8.0	FR infant	7.1	Carrots	0.6	Turnips	0.3	Celeriac	
7.9	FR toddler	6.5	Carrots	0.7	Turnips	0.3	Celeriac	
4.5	IE adult	1.7	Parsnips	0.9	Carrots	0.7	Swedes	
4.0	UK Infant	3.5	Carrots	0.3	Swedes	0.1	Parsnips	
3.8	DK child	3.7	Carrots	0.1	Celeriac	0.0	Beetroot	
3.5	SE general population 90th percentile	2.3	Carrots	0.2	Beetroot	0.2	Beetroot	
3.1	DE child	2.7	Carrots	0.1	Celeriac	0.0	Beetroot	
3.0	WHO Cluster diet B	1.8	Beetroot	0.7	Carrots	0.3	Parsnips	
2.5	WHO Cluster diet F	1.3	Carrots	0.6	Swedes	0.6	Swedes	
2.0	NL child	1.3	Carrots	0.4	Beetroot	0.0	Celeriac	
1.8	PT General population	1.8	Carrots	0.0	Turnips		FRUIT (FRESH OR FROZEN)	
1.7	PL general population	0.8	Carrots	0.6	Beetroot	0.2	Parsley root	
1.6	WHO cluster diet E	1.2	Carrots	0.3	Beetroot	0.1	Radishes	
1.6	UK Toddler	1.4	Carrots	0.1	Swedes	0.1	Turnips	
1.4	WHO regional European diet	1.0	Carrots	0.1	Radishes	0.1	Beetroot	
1.3	DK adult	1.2	Carrots	0.1	Celeriac	0.0	Beetroot	
1.2	WHO cluster diet D	0.6	Carrots	0.2	Radishes	0.1	Horseradish	
1.1	FR all population	0.8	Carrots	0.1	Radishes	0.1	Turnips	
0.9	NL general	0.6	Carrots	0.2	Beetroot	0.0	Celeriac	
0.8	LT adult	0.5	Carrots	0.3	Beetroot	0.0	Horseradish	
0.8	UK vegetarian	0.6	Carrots	0.1	Beetroot	0.0	Swedes	
0.7	FI adult	0.5	Carrots	0.1	Beetroot	0.1	Swedes	
0.6	UK Adult	0.5	Carrots	0.0	Swedes	0.0	Parsnips	
0.5	ES child	0.5	Carrots	0.0	Other other root and tuber vegetables	0.0	Eggs: Chicken	
0.5	IT kids/toddler	0.5	Carrots	0.0	Turnips	0.0	Celeriac	
0.4	IT adult	0.4	Carrots	0.0	Beetroot	0.0	Turnips	
0.4	ES adult	0.4	Carrots	0.0	Other other root and tuber vegetables	0.0	Beetroot	

Conclusion:
The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of RP44247 is unlikely to present a public health concern.

Table 6.9-28: TMDI calculation for DCHPU with PRIMo Model (rev 2.0)

DCPHU

Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0.0015	ARfD (mg/kg bw):	
Source of ADI:	TTC	Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Prepare workbook for refined calculations

Undo refined calculations

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

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)
0.1	WHO Cluster diet B	0.1	Bovine: Kidney	0.0	Bovine: Meat	0.0	Bovine: Liver	
0.0	NL child	0.0	Bovine: Meat	0.0	Bovine: Liver	0.0	Bovine: Kidney	
0.0	UK infant	0.0	Bovine: Kidney	0.0	Bovine: Liver		FRUIT (FRESH OR FROZEN)	
0.0	ES child	0.0	Bovine: Meat	0.0	Bovine: Liver	0.0	Swine: Liver	
0.0	WHO cluster diet D	0.0	Bovine: Kidney	0.0	Bovine: Meat	0.0	Bovine: Liver	
0.0	WHO Cluster diet F	0.0	Bovine: Meat	0.0	Bovine: Kidney	0.0	Bovine: Liver	
0.0	DK child	0.0	Bovine: Liver		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
0.0	FR toddler	0.0	Bovine: Meat		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
0.0	WHO regional European diet	0.0	Bovine: Meat	0.0	Bovine: Kidney	0.0	Bovine: Liver	
0.0	DK adult	0.0	Bovine: Liver	0.0	Bovine: Meat		FRUIT (FRESH OR FROZEN)	
0.0	NL general	0.0	Bovine: Meat	0.0	Bovine: Liver	0.0	Swine: Liver	
0.0	WHO cluster diet E	0.0	Bovine: Meat	0.0	Bovine: Liver	0.0	Bovine: Fat	
0.0	ES adult	0.0	Bovine: Meat	0.0	Bovine: Liver	0.0	Swine: Liver	
0.0	UK Toddler	0.0	Bovine: Kidney	0.0	Bovine: Liver		FRUIT (FRESH OR FROZEN)	
0.0	FR infant	0.0	Bovine: Meat		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
0.0	FR all population	0.0	Bovine: Meat		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
0.0	LT adult	0.0	Bovine: Meat	0.0	Bovine: Liver	0.0	Swine: Liver	
0.0	IE adult	0.0	Bovine: Meat		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
0.0	DE child	0.0	Bovine: Meat		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
0.0	UK Adult	0.0	Bovine: Kidney	0.0	Bovine: Liver		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 DCPHU is unlikely to present a public health concern.

Table 6.9-29: TMDI calculation for M610F007 with PRIMo Model (rev 2.0)

M610F007			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0.0015	ARfD (mg/kg bw):	
Source of ADI:	TTC	Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Prepare workbook for refined calculations

Undo refined calculations

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	
4	67
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)
67.1	FR infant	67.1	Carrots		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
61.9	FR toddler	61.9	Carrots		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
34.8	DK child	34.8	Carrots		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
33.5	UK infant	33.5	Carrots		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
26.1	DE child	26.0	Carrots	0.0	Lamb's lettuce		FRUIT (FRESH OR FROZEN)	
21.5	SE general population 90th percentile	21.5	Carrots		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
16.9	PT General population	16.9	Carrots		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
13.2	UK Toddler	13.2	Carrots		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
12.7	NL child	12.7	Carrots	0.0	Lamb's lettuce		FRUIT (FRESH OR FROZEN)	
12.0	WHO Cluster diet F	12.0	Carrots		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
11.4	WHO cluster diet E	11.4	Carrots		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
11.3	DK adult	11.3	Carrots		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
9.3	WHO regional European diet	9.3	Carrots		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
8.1	IE adult	8.1	Carrots		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
7.7	PL general population	7.7	Carrots		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
7.6	FR all population	7.6	Carrots	0.0	Lamb's lettuce		FRUIT (FRESH OR FROZEN)	
6.4	WHO Cluster diet B	6.4	Carrots		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
5.9	WHO cluster diet D	5.9	Carrots		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
5.7	UK vegetarian	5.7	Carrots		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
5.5	NL general	5.5	Carrots	0.0	Lamb's lettuce		FRUIT (FRESH OR FROZEN)	
4.8	FI adult	4.8	Carrots		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
4.7	IT kids/toddler	4.6	Carrots	0.0	Lamb's lettuce		FRUIT (FRESH OR FROZEN)	
4.6	UK Adult	4.6	Carrots		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
4.5	ES child	4.5	Carrots		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
4.4	LT adult	4.4	Carrots		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
3.6	IT adult	3.6	Carrots	0.0	Lamb's lettuce		FRUIT (FRESH OR FROZEN)	
3.6	ES adult	3.6	Carrots		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 M610F007 is unlikely to present a public health concern.

Table 6.9-30: TMDI calculation for RP37176 with VELS Model

Active substance:	RP37176	Total intake in mg/kg bw:	0.000199034
ADI (mg/kg bw):	0.0015	% ADI	13.26893705
Mean bodyweight (kg):	16.15	Calculation method:	TMDI

Code number	Commodity	chronic			
		Mean consumption (g/d)	MRL (mg/kg)	STMR (mg/kg)	Intake (mg/kg bw)
0130010	Apples, processed	6.8	0.14		5.89474E-05
0231010	Tomatoes, processed	10.3	0.08		5.10217E-05
0152000	Strawberries, processed	1	0.3		1.85759E-05
0231020	Peppers, processed	1.5	0.16		1.48607E-05
0140030	Peaches and nectarines, processed	1.4	0.1		8.66873E-06
0153030	Raspberries, processed	0.4	0.3		7.43034E-06
0213020	Carrots, processed	6.9	0.016		6.83591E-06
0232010	Cucumber, processed	1.6	0.06		5.94427E-06
0154010	Blueberries, processed	0.2	0.3		3.71517E-06
0154030	Currants, processed	0.2	0.3		3.71517E-06
0232030	Courgette, processed	0.9	0.06		3.34365E-06
0130020	Pears, processed	0.3	0.14		2.60062E-06
0251000	a) Lettuce and other salad plants including Brassicacea	1.4	0.03		2.60062E-06
0153010	Blackberries, processed	0.1	0.3		1.85759E-06
0154040	Gooseberries , processed*	0.1	0.3		1.85759E-06
0140040	Plums, processed	0.6	0.03		1.11455E-06
0241020	Cauliflower, processed	2.1	0.008		1.04025E-06
0241010	Broccoli, processed	1.8	0.008		8.91641E-07
0220020	Onions, processed	2.7	0.004		6.68731E-07
0243010	Chinese cabbage, processed	0.1	0.1		6.19195E-07
0231030	Egg plant, processed	0.1	0.08		4.95356E-07
0233020	Squash/Pumpkins, processed	0.2	0.03		3.71517E-07
0256010	Chervil, raw*	0.1	0.03		1.85759E-07
0256020	Chives, raw	0.1	0.03		1.85759E-07
0256030	Dill, raw*	0.1	0.03		1.85759E-07
0256040	Parsley, raw	0.1	0.03		1.85759E-07
0256050	Sage, raw*	0.1	0.03		1.85759E-07
0256060	Rosemary, raw*	0.1	0.03		1.85759E-07
0256070	Thyme, raw*	0.1	0.03		1.85759E-07
0256080	Basil, raw	0.1	0.03		1.85759E-07
0256990	Oregano, raw*	0.1	0.03		1.85759E-07
0270010	Asparagus, processed	0.3	0.004		7.43034E-08
0260030	Peas, processed*	0.1	0.006		3.71517E-08
0220010	Garlic, processed	0.1	0.004		2.47678E-08
0220030	Shallot, processed*	0.1	0.004		2.47678E-08
0300020	Lentils dry, processed	0.1	0.004		2.47678E-08

Acute Reference Dose (ARfD) and Dietary Exposure Calculation

NESTI calculations

NESTI calculations are not required at this point in time. No ARfD has been allocated to iprodione as it was not considered necessary.

CA 6.10 Other studies

No other/special studies were deemed necessary. The studies and information provided under previous sections are considered adequate and sufficient.

CA 6.10.1 Effect on the residue level in pollen and bee products

The objective of these studies shall be to determine the residue in pollen and bee products for human consumption resulting from residues taken up by honeybees from crops at blossom.

As the representative uses of this dossier are carrots and lettuce, pollen and bees are not an issue.

Tier 1 Summaries of the Supervised Field Residue Trials

Carrots

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name):	**iprodione**	**Commercial product:**	
Crop /crop group:	**Carrot/ root vegetable**	**Producer of commercial product:**	
Responsible body for reporting	**BASF AG, 67056 Ludwigshafen, Germany**	**Indoor/outdoor**	**Outdoor**
Country (trial)	**Germany**	**Other a.i. in the formulation**	**-**
Content of active substance (g/kg or g/L):	**500 g/kg**	**Content:**	
Formulation	**EXP01862F (SC)**	**Residue calculated as:**	**iprodione**

Report No Date of delivery	Location incl. Post code Country	Variety / Date of 1) Sowing or planting 2) Flowering 3) Harvest	Rate of treatment a.i. (kg a.s./ha)	 Water (l/ha)	 a.i. (kg a.s./hL)	Number of treatments and last date	Portion of commodity analysed	PHI (days)	Residues (mg/kg)	Remarks
C025546 98-750 R&D/CRLD/AN/kd/9916135 18-August-99	98750RN1 (Decline study) France North	Major 1) 06/06/98	0.732 0.732 0.732	333 333 333	0.220 0.220 0.220	17/08/98 (1) 31/08/98 (2) 16/09/98 (3)	root root	26	0.17	Residues were less than LOQ in untreated samples Method of analysis : AR144-97 modified LOQ **:** 0.020 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Carrots (Root and tuber vegetables)	**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**	-
Content of active substance (g/kg or g/L)	750 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 610 06 F)	**Residues calculated as:**	Iprodione (I[2]) RP 30228 (II[3])and RP 32490 (III[4]) both as parent equiv., RP 32596 (IV[5]), RP 37176 (V[6]) and M610F007 (VI[7]) as metabolites

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 per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH) at Last Treat.**	**Portion Analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I[2]**	**II[3]**	**III[4]**	**IV[5]**	**V[6]**	**VI[7]**		
384192 2011/1120989 67125 Dannstadt Germany L100668	Carrot Maestro (Niz/Vil)	1. 26.06.2010 2. - 3. 28.11.2010	spray	0.150	500	0.75	4 12.10.2010	48	whole plant* roots tops roots tops roots tops	8.29 0.18 11.50 0.20 6.42 0.15 6.25	0.02 <0.01 0.08 <0.01 0.08 <0.01 0.10	0.01 <0.01 0.09 <0.01 0.07 <0.01 0.08	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	- - - - - - -	- 0.28 - 0.24 - 0.29 -	0 22 22 28 28 35 35	Method L0180/01 LOQ 0.01 mg/kg
384192 2011/1120989 6598 Heljen The Netherlands L100238	Carrot Maxi	1. 22.04.2010 2. - 3. 07.09.2010	spray	0.150	500	0.75	4 03.08.2010	45	whole plant* roots tops roots tops roots tops	4.48 0.14 2.16 0.12 1.54 0.12 0.80	0.05 0.02 0.21 0.01 0.16 0.01 0.08	<0.01 <0.01 0.02 <0.01 0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	- - - - - - -	- 0.07 - 0.08 - 0.04 -	0 20 20 28 28 35 35	Method L0180/01 LOQ 0.01 mg/kg
383567 2011/1120989 02820 Aisne France (North) L100239	Carrot Karotan	1. 05.05.2010 2. - 3. 27.10.2010	spray	0.150	500	0.75	4 11.10.2010	48	whole plant* roots tops roots tops roots tops	5.84 0.07 3.28 0.09 4.04 0.05 2.38	0.03 <0.01 0.06 <0.01 0.12 <0.01 0.10	<0.01 <0.01 0.02 <0.01 0.03 <0.01 0.02	<0.01 <0.01 <0.01 <0.01 0.02 <0.01 0.01	- - - - - - -	- 0.32 -- 0.32 - 0.26 	0 22 22 26 26 35 35	Method L0180/01 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Carrots (Root and tuber vegetables)	**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**	-
Content of active substance (g/kg or g/L)	750 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 610 06 F)	**Residues calculated as:**	Iprodione (I[2]) RP 30228 (II[3])and RP 32490 (III[4]) both as parent equiv., RP 32596 (IV[5]), RP 37176 (V[6]) and M610F007 (VI[7]) as metabolites

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 per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH) at Last Treat.**	**Portion Analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I[2]**	**II[3]**	**III[4]**	**IV[5]**	**V[6]**	**VI[7]**		
383567 2011/1120989 Clumber / Nottinghamshire United Kingdom L100240	Carrot Nairobi	1. 27.04.2010 2. - 3. 29.10.2010	spray	0.150	500	0.75	4 22.09.2010	47	whole plant* roots tops roots tops roots tops	5.35 0.09 4.24 0.09 5.60 0.09 6.48	0.01 <0.01 0.20 <0.01 0.20 <0.01 0.33	0.02 <0.01 0.10 <0.01 0.08 <0.01 0.11	<0.01 <0.01 0.01 <0.01 0.01 <0.01 <0.01	 - - - - - -	 0.14 - 0.35 - 0.19 -	0 21 21 28 28 35 35	Method L0180/01 LOQ 0.01 mg/kg
384194 2013/1106641 6562 KC Groesbeek/Gelderland The Netherlands L110383	Carrot Komamo F1	1. 11.05.2011 2. - 3. 19.09.-26.09.2011	spray	0.150	500	0.75	4 22.08.2011	45	whole plant* roots tops roots tops roots tops	11.0 0.16 0.88 0.08 0.98 0.08 0.67	0.05 <0.01 0.05 <0.01 0.06 <0.01 0.06	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	- 0.10 - 0.11 - 0.06 -	0 21 21 28 28 35 35	Method L0180/01 LOQ 0.01 mg/kg
384194 2013/1106641 79356 Eichstetten Germany L110384	Carrot Bolero	1. 10.06.2011 2. - 3. 08.09.2011	spray	0.150	500	0.75	4 10.08.2011	41-43	whole plant* roots tops roots tops roots tops	11.0 0.12 3.10 0.08 2.30 0.07 2.10	0.04 <0.01 0.09 <0.01 0.03 <0.01 0.06	0.01 <0.01 0.05 <0.01 0.03 <0.01 0.03	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	- 0.05 - 0.05 - 0.04 -	0 21 21 26 26 34 34	Method L0180/01 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Carrots (Root and tuber vegetables)	**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**	-
Content of active substance (g/kg or g/L)	750 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 610 06 F)	**Residues calculated as:**	Iprodione (I[2]) RP 30228 (II[3])and RP 32490 (III[4]) both as parent equiv., RP 32596 (IV[5]), RP 37176 (V[6]) and M610F007 (VI[7]) as metabolites

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 per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH) at Last Treat.**	**Portion Analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I[2]**	**II[3]**	**III[4]**	**IV[5]**	**V[6]**	**VI[7]**		
384194 2013/1106641 67117 Handschuheim France L110385	Carrot Bolero	1. 18.05.2011 2. - 3. 20.09.-15.10.2011	spray	0.150	500	0.75	4 31.08.2011	45-47	whole plant* roots tops roots tops roots tops	2.80 0.07 2.00 0.08 1.80 0.08 1.60	0.04 <0.01 0.09 <0.01 0.09 <0.01 0.06	<0.01 <0.01 0.02 <0.01 0.01 <0.01 0.02	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	- 0.20 - 0.19 - 0.16 -	0 22 22 29 29 35 35	Method L0180/01 LOQ 0.01 mg/kg
384194 2013/1106641 Clumber, Nottinghamshire United Kingdom L110386	Carrot Newark	1. 29.04.2011 2. - 3. 2012	spray	0.150	500	0.75 **	4 13.10.2011	43-45	whole plant* roots tops roots tops roots tops	4.40 0.08 22.00 0.06 16.00 0.03 6.80	0.04 <0.01 1.00 <0.01 1.10 <0.01 3.80	<0.01 <0.01 0.07 <0.01 0.06 <0.01 0.08	0.02 <0.01 0.05 <0.01 0.06 <0.01 0.43	<0.01 <0.01 <0.01 <0.01 0.01 <0.01 0.02	- 0.46 - 0.38 - 0.25 -	0 20 20 27 27 35 35	Method L0180/01 LOQ 0.01 mg/kg

0) actual application rates varied by 10% at most
1) days after last application
2) parent BAS 610 F
3) RP 30228 (Reg. No. 5079647), expressed as parent equivalent, conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 32490 (Reg. No. 5079628), expressed as parent equivalent, conversion factor from metabolite to parent BAS 610 F is 1.146
5) RP 32596 (Reg. No. 85831)
6) RP 37176 (Reg. No. 5079612)
7) M610F007 (Reg. No. 5916256)

* without roots

** erroneously, a treatment with COMPASS (BAS 613 01 F) was done, resulting in an additional rate of 225 g iprodione applied between 3rd and 4th regular treatment. The results in tops should not be taken into account in any evaluation.

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name):	iprodione	**Commercial product:**	
Crop /crop group:	Carrot/ root vegetable	**Producer of commercial product:**	
Responsible body for reporting	BASF AG, 67056 Ludwigshafen, Germany	**Indoor/outdoor**	Outdoor
Country (trial)	Germany	**Other a.i. in the formulation**	-
Content of active substance (g/kg or g/L):	500 g/kg	**Content:**	
Formulation	SC (BAS 610 05 F)	**Residue calculated as:**	iprodione

Report No Date of delivery	Location incl. Post code Country	Variety / Date of 1) Sowing or planting 2) Flowering 3) Harvest	Rate of treatment a.i. (kg a.s./ha)	 Water (l/ha)	 a.i. (kg a.s./hL)	Number of treatments and last date	Portion of commodity analysed	PHI (days)	Residues (mg/kg)	Remarks
2004/7007471	Andalucia Spain	1) 06.05.04	0.150	500	0.750	30.06.04 09.07.04 19.07.04 29.07.04	whole plant root root root	0 21 27 35	38.65 0.10 0.11 0.08	Analytical method: BASF method No.: 453/0
	Midi-Pyrenees France (S)	1) 27.05.04	0.150	500	0.750	20.07.04 30.07.04 09.08.04 19.08.04	whole plant root root root	0 21 28 35	13.22 0.15 0.16 0.18	Analytical method: BASF method No.: 453/0
	Macedonia Greece	1) 28.06.04	0.150	500	0.750	02.09.04 11.09.04 22.09.04 02.10.04	whole plant root root root	0 20 27 34	19.46 0.38 0.30 0.22	Analytical method: BASF method No.: 453/0
	Piemonte Italy	1) 15.03.04	0.150	500	0.750	28.05.04 07.06.04 17.06.04 28.06.04	whole plant root root root	0 21 28 35	5.64 0.24 0.15 0.13	Analytical method: BASF method No.: 453/0
2005/1004980	Trajano Sevilla Spain	1) 10.10.03 3) 10.-15.03.04	0.150	500	0.750	4 06.02.04	whole plant root root root	0 21 28 35	11.68 0.42 0.46 0.39	Analytical method BASF method No M 543/0 LOQ 0.01 mg/kg
	El Cuervo Sevilla Spain	1) 14.10.03 3) 12.-19.03.04	0.150	500	0.750	4 06.02.04	whole plant root root root	0 21 28 35	9.12 0.40 0.40 0.37	Analytical method BASF method No M 543/0 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name):	iprodione	**Commercial product:**	
Crop /crop group:	Carrot/ root vegetable	**Producer of commercial product:**	
Responsible body for reporting	BASF AG, 67056 Ludwigshafen, Germany	**Indoor/outdoor**	Outdoor
Country (trial)	Germany	**Other a.i. in the formulation**	-
Content of active substance (g/kg or g/L):	500 g/kg	**Content:**	
Formulation	SC (BAS 610 05 F)	**Residue calculated as:**	iprodione

Report No Date of delivery	Location incl. Post code Country	Variety / Date of 1) Sowing or planting 2) Flowering 3) Harvest	Rate of treatment a.i. (kg a.s./ha)	 Water (l/ha)	 a.i. (kg a.s./hL)	Number of treatments and last date	Portion of commodity analysed	PHI (days)	Residues (mg/kg)	Remarks
	St. Paul les Romans France (S)	1) 25.06.03 3) 24.-28.09.04	0.150	500	0.750	4 28.08.04	whole plant root root root	0 21 28 35	18.94 0.05 0.04 0.03	Analytical method BASF method No M 543/0 LOQ 0.01 mg/kg
	Tortona, Cascina, Viscarda, Italy	1) 05.03.03 3) 20.07.-20.08.04	0.150	500	0.750	4 30.06.04	whole plant root root root	0 21 28 35	8.08 0.31 0.23 0.23	Analytical method BASF method No M 543/0 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Carrots (Root and tuber vegetables)	**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**	-
Content of active substance (g/kg or g/L)	750 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 610 06 F)	**Residues calculated as:**	Iprodione (I^2) RP 30228 (II^3)and RP 32490 (III^4) both as parent equiv., RP 32596 (IV^5), RP 37176 (V^6) and M610F007 (VI^7) as metabolites

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 per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH) at Last Treat.**	**Portion Analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I^2**	**I^3**	**III^4**	**IV^5**	**V^6**	**VI^7**		
383567 2011/1120989 30210 Gard / Sernac France (South) L100241	Carrot Maestro	1. 07.07.2010 2. - 3. 05.11.2010	spray	0.150	500	0.75	4 06.10.2010	44	whole plant* roots tops roots tops roots tops	8.08 0.26 4.84 0.31 3.92 0.14 2.32	0.03 0.01 0.05 0.02 0.08 0.01 0.06	0.02 <0.01 0.08 <0.01 0.07 <0.01 0.04	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	- - - - - - -	- 0.12 - 0.11 - 0.25 -	0 21 21 27 27 35 35	Method L0180/01 LOQ 0.01 mg/kg
383567 2011/1120989 57007 Tessaloniki Greece L100242	Carrot Tempo F1	1. 10.06.2010 2. - 3. 20.09.2010	spray	0.150	500	0.75	4 20.08.2010	44	whole plant* roots tops roots tops roots tops	35.28 0.34 9.62 0.27 8.88 0.33 8.36	0.10 0.02 0.17 0.02 0.17 0.03 0.19	0.08 <0.01 0.10 <0.01 0.09 <0.01 0.07	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	- - - - - - -	- 0.04 - 0.05 - 0.08 -	0 21 21 27 27 35 35	Method L0180/01 LOQ 0.01 mg/kg
383567 2011/1120989 12050 Barbaresco Italy L100243	Carrot Nador	1. 28.07.2010 2. - 3. 15.10.2010	spray	0.150	500	0.75	4 17.09.2010	43-45	whole plant* roots tops roots tops roots tops	8.19 0.29 5.54 0.35 5.43 0.36 5.04	0.12 0.03 0.20 0.04 0.16 0.04 0.18	0.02 <0.01 0.05 <0.01 0.04 <0.01 0.04	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	- - - - - - -	- - - - - - -	0 21 21 27 27 35 35	Method L0180/01 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Carrots (Root and tuber vegetables)	**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**	-
Content of active substance (g/kg or g/L)	750 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 610 06 F)	**Residues calculated as:**	Iprodione (I^2) RP 30228 (II^3)and RP 32490 (III^4) both as parent equiv., RP 32596 (IV^5), RP 37176 (V^6) and M610F007 (VI^7) as metabolites

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 per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH) at Last Treat.**	**Portion Analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I^2**	**I^3**	**III^4**	**IV^5**	**V^6**	**VI^7**		
383567 2011/1120989 11540 Sanlucar de Barrameda Spain L100244	Carrot Nipo	1. 02.09.2010 2. - 3. 17.12.2010	spray	0.150	500	0.75	4 12.11.2010	43	whole plant*	7.26	0.03	0.02	<0.01	-	-	0	Method
									roots	0.11	0.01	<0.01	<0.01	-	-	20	L0180/01
									tops	2.06	0.04	0.03	<0.01	-	-	20	LOQ
									roots	0.12	0.02	<0.01	<0.01	-	-	27	0.01 mg/kg
									tops	2.12	0.06	0.02	<0.01	-	-	27	
									roots	0.09	0.01	<0.01	<0.01	-	-	34	
									tops	1.54	0.04	0.02	<0.01	-	-	34	
384194 2013/1106641 31330 Ondes France (South) L110387	Carrot Bolero	1. 20.06.2011 2. - 3. 05.10.-07.10.2011	spray	0.150	500	0.75	4 09.09.2011	45	whole plant*	10.00	0.06	<0.01	<0.01	0.01	-	0	Method
									roots	0.17	0.02	<0.01	<0.01	<0.01	0.11	21	L0180/01
									tops	2.40	0.10	0.02	<0.01	<0.01	-	21	LOQ
									roots	0.15	0.02	<0.01	<0.01	<0.01	0.08	27	0.01 mg/kg
									tops	1.20	0.08	0.01	<0.01	<0.01	-	27	
									roots	0.11	0.02	<0.01	<0.01	<0.01	0.06	35	
									tops	1.40	0.07	0.02	<0.01	<0.01	-	35	
384194 2013/1106641 5701 Prochoma Greece L110388	Carrot Entopio	1. 22.07.2011 2. - 3. 10.11.-25.11.2011	spray	0.150	500	0.75	4 17.10.2011	45-46	whole plant*	8.40	0.04	<0.01	<0.01	<0.01	-	0	Method
									roots	0.13	0.01	<0.01	<0.01	<0.01	0.01	21	L0180/01
									tops	3.30	0.19	0.05	0.01	<0.01	-	21	LOQ
									roots	0.11	0.01	<0.01	<0.01	<0.01	0.01	26	0.01 mg/kg
									tops	1.90	0.02	0.03	<0.01	<0.01	-	26	
									roots	0.11	0.01	<0.01	<0.01	<0.01	0.01	35	
									tops	2.70	0.15	0.05	<0.01	<0.01	-	35	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Carrots (Root and tuber vegetables)	**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**	-
Content of active substance (g/kg or g/L)	750 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 610 06 F)	**Residues calculated as:**	Iprodione (I^2) RP 30228 (II^3)and RP 32490 (III^4) both as parent equiv., RP 32596 (IV^5), RP 37176 (V^6) and M610F007 (VI^7) as metabolites

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 per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH) at Last Treat.**	**Portion Analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I^2**	**I^3**	**III^4**	**IV^5**	**V^6**	**VI^7**		
384194 2013/1106641 15059 Castelnuovo Scrivia Italy L110389	Carrot Ceres	1. 10.04.2011 2. - 3. 01.09.-10.09.2011	spray	0.150	500	0.75	4 05.08.2011	47	whole plant* roots tops roots tops roots tops	6.10 0.46 33.00 0.45 21.00 0.46 12.00	0.07 0.05 0.31 0.05 0.31 0.09 0.85	0.03 <0.01 0.50 <0.01 0.33 <0.01 0.28	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 0.02	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	- 0.11 - 0.16 - 0.23 -	0 21 21 29 29 35 35	Method L0180/01 LOQ 0.01 mg/kg
384194 2013/1106641 08380 Malgrat De Mar Spain L110390	Carrot Evora	1. 25.08.2011 2. - 3. 27.10.-05.11.2011	spray	0.150	500	0.75	4 30.09.2011	42	whole plant* roots tops roots tops roots tops	32.00 0.25 2.30 0.15 2.10 0.09 1.70	0.08 0.02 0.08 0.01 0.08 0.01 0.06	0.01 <0.01 0.03 <0.01 0.02 <0.01 <0.01	0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	- 0.02 - 0.01 - 0.01 -	0 20 20 27 27 34 34	Method L0180/01 LOQ 0.01 mg/kg

0) actual application rates varied by 10% at most
1) days after last application
2) parent BAS 610 F
3) RP 30228 (Reg. No. 5079647), expressed as parent equivalent, conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 32490 (Reg. No. 5079628), expressed as parent equivalent, conversion factor from metabolite to parent BAS 610 F is 1.146
5) RP 32596 (Reg. No. 85831)
6) RP 37176 (Reg. No. 5079612)
7) M610F007 (Reg. No. 5916256)
* without roots

Lettuce

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Lettuce (group 6.3.11 Leafy vegetables)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Greenhouse
Country	Germany	**Other active substance in the formulation**	-
Content of active substance (g/kg or g/L)	500 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WP (EXP01671H)	**Residues calculated as:**	Iprodione (BAS 610)

1	2	3	4	5			6	7	8	9	10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Portion Analysed**	**Residues (mg/kg)***	**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I[3]**		
99-634	Sensai	1. 08.09.1999	spray	0125	600	0.7500	3	48	leaf	7.6	0	Method
R018833		2. 15./22.10.1999		0.131	600	0.7855	15.10.1999			4.6	7	No AR144-97
67366 Weingarten		3. 29.10.1999		0.132	600	0.7910				3.4	14	LOQ
Germany												0.02 mg/kg
99634DE-1												

0) actual application rates varied by 10% at most
1) days after last application
2) at treatment time
3) parent BAS 610 F

* Residues were between 0.11 mg/kg (DALA = 0 day) and 0.030 mg/kg (DALA = 14 days) in untreated samples.

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Lettuce (group 6.3.11 Leafy vegetables)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Greenhouse
Country	Germany	**Other active substance in the formulation**	-
Content of active substance (g/kg or g/L)	750 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 610 06 F)	**Residues calculated as:**	Iprodione (I^2) RP 30228 (II^3)and RP 32490 (III^4) both as parent equiv., RP 32596 (IV^5), RP 37176 (V^6) and M610F007 (VI^7) as metabolites

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 per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH) at Last Treat.**	**Portion Analysed**	**Residues (mg/kg)**							**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I^2**	**II^3**	**III^4**	**IV^5**	**V^6**	**VI^7**			
384191	VL 0482	1. 28.08.2013	spray	0.15	500	0.750	3	47	head**	24	0.14	0.051	<0.01	<0.01	<0.01	0	Method
2014/1094138	Lolo Bionda	2. -		0.15	500	0.750	25.09.2013		head**	19	0.10	0.049	<0.01	<0.01	<0.01	7	L0180/01
04827 Gerichshain	(Bartimer)	3. 08.10.2013		0.15	500	0.750			head**	15	0.081	0.052	<0.01	<0.01	<0.01	13	LOQ
Germany									head**	3.9	0.020	0.018	<0.01	<0.01	<0.01	21	0.01 mg/kg
L130585																	
384191	VL 0482	1. 30.09.2013	spray	0.15	500	0.750	3	43	head**	19	0.10	0.018	<0.01	<0.01	<0.01	0	Method
2014/1094138	Lolo livorno	2. -		0.15	500	0.750	29.10.2013		head**	8.9	0.57	0.072	0.010	<0.01	<0.01	7	L0180/01
37340 Ambillou		3. 12.11.2013		0.15	500	0.750			head**	4.1	0.28	0.061	<0.01	<0.01	<0.01	14	LOQ
France (North)									head**	0.58	0.093	0.028	<0.01	<0.01	<0.01	21	0.01 mg/kg
L130586																	
384191	VL 4357	1. 12.08.2013	spray	0.15	500	0.750	3	46	wh plant*	53	0.52	0.031	<0.01	0.016	0.015	0	Method
2014/1094138	Gala	2. -		0.15	500	0.750	25.09.2013		wh plant*	20	0.96	0.053	<0.01	0.016	0.032	7	L0180/01
04668 Motterwitz	Lamb's lettuce	3. 08.10.2013		0.15	500	0.750			wh plant*	11	0.58	0.050	0.019	<0.01	0.048	13	LOQ
Germany									wh plant*	9.3	0.52	0.054	0.026	<0.01	0.080	21	0.01 mg/kg
L130591																	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Lettuce (group 6.3.11 Leafy vegetables)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Greenhouse
Country	Germany	**Other active substance in the formulation (common name and content)**	-
Content of active substance (g/kg or g/L)	750 g/kg		
Formulation (e.g. WP)	WG (BAS 610 06 F)	**Residues calculated as:**	Iprodione (I[2]) RP 30228 (II[3])and RP 32490 (III[4]) both as parent equiv., RP 32596 (IV[5]), RP 37176 (V[6]) and M610F007 (VI[7]) as metabolites

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 per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH) at Last Treat.**	**Portion Analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I[2]**	**II[3]**	**III[4]**	**IV[5]**	**V[6]**	**VI[7]**		
384191	VL 0482	1. 08.07.2013	spray	0.15	500	0.750	3	41	wh plant*	21	2.0	0.039	<0.01	0.028	0.013	0	Method
2014/1094138	Trophy	2. -		0.15	500	0.750	15.08.2013		wh plant*	8.8	2.0	0.038	0.010	0.027	0.028	7	L0180/01
5853 AB Siebengewald The Netherlands	Lamb's lettuce	3. 28.08.2013		0.15	500	0.750			wh plant*	4.6	1.0	0.034	<0.01	0.014	0.016	13	LOQ
L130592									wh plant*	0.88	0.26	<0.01	<0.01	<0.01	<0.010	21	0.01 mg/kg

0) actual application rates varied by 10% at most
1) days after last application
2) parent BAS 610 F
3) RP 030228 (Reg. No. 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 032490 (Reg. No. 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
5) RP 032596 (Reg. No. 85831)
6) RP 037176 (Reg. No. 5079612)
7) M610F007 (Reg. No. 5916256)

* without roots ** open leaf varieties

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Lettuce (group 6.3.11 Leafy vegetables)	**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)**	-
Content of active substance (g/kg or g/L)	500 g/L		
Formulation (e.g. WP)	SC (EXP01862F)	**Residues calculated as:**	Iprodione (BAS 610)

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 per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Portion Analysed**	**Residues (mg/kg)**	**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I[3]**		
CA 6.3.2/3 (99-695) R018826 Thorrington, Essex United Kingdom 99695GB1	Little Gem ev Swing	1. 11.08.1999 2. - 3. 30.09.1999	spray	0.118 0.108 0.117	662 662 662	0.782 0.717 0.772	3 09.09.1999	19	leaf	0.025	21	Method No AR144-97 (modified) LOQ 0.02 mg/kg
CA 6.3.2/3 (99-695) R018826 Brentwood, Essex United Kingdom 99695GB2	Little Gem ev Swing	1. 12.08.1999 2. - 3. 01.10.1999	spray	0.117 0.113 0.111	662 662 662	0.777 0.749 0.737	3 10.09.1999	19	leaf	0.11*	21	Method No AR144-97 (modified) LOQ 0.02 mg/kg

0) actual application rates varied by 10% at most
1) days after last application
2) at treatment time
3) BAS 610 F

* An inversion was suspected between the untreated samples (1R2; 0.11 mg/kg) and between the treated samples (2R1; <0.020 mg/kg) for the trial 99695GB2. The value obtained for 1R2 was taken into account.

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Lettuce (group 6.3.11 Leafy vegetables)	**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)**	-
Content of active substance (g/kg or g/L)	750 g/kg		
Formulation (e.g. WP)	WG (BAS 610 06 F)	**Residues calculated as:**	Iprodione (I^2) RP 30228 (II^3)and RP 32490 (III^4) both as parent equiv., RP 32596 (IV^5), M610F007 (VI^6)

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 per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH) at Last Treat.**	**Portion Analysed**	**Residues (mg/kg)**					**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I^2**	**II^3**	**III^4**	**IV^5**	**VI^6**		
384189	VL0483	1. 04.05.2010	spray	0.15	500	0.750	3	35	wh. plant*	13.14	0.03	0.03	<0.01	-	0	Method
2011/1120988	Lollo Bionda	2. -		0.15	500	0.750	27.05.2010		head	0.32	0.02	0.03	<0.01	<0.01	14	L0180/01
67245 Lambsheim		3. 20.06.2010		0.15	500	0.750			head	0.01	<0.01	<0.01	<0.01	<0.01	21	LOQ
Germany									head	<0.01	<0.01	<0.01	<0.01	<0.01	28	0.01 mg/kg
L100245																
384189	VL0483	1. 20.05.2010	spray	0.15	500	0.750	3	41	wh. plant*	11.34	0.05	0.05	<0.01	-	0	Method
2011/1120988	Kyrio	2. -		0.15	500	0.750	30.06.2010		head	1.21	0.12	0.08	<0.01	<0.01	13	L0180/01
80600 Mezerolles		3. 12.07.2010		0.15	500	0.750			head	0.04	<0.01	0.01	<0.01	<0.01	21	LOQ
France									head	<0.01	<0.01	<0.01	<0.01	<0.01	28	0.01 mg/kg
L100246																
384189	VL0483	1. 05.07.2010	spray	0.15	500	0.750	3	45	wh. plant*	10.94	0.03	<0.01	<0.01	-	0	Method
2011/1120988	Lollo Biance	2. -		0.15	500	0.750	12.08.2010		head	0.04	<0.01	<0.01	<0.01	<0.01	14	L0180/01
3545 Limburg		3. 25.08.2010		0.15	500	0.750			head	<0.01	<0.01	<0.01	<0.01	<0.01	21	LOQ
Belgium									head	<0.01	<0.01	<0.01	<0.01	<0.01	28	0.01 mg/kg
L100247																

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Lettuce (group 6.3.11 Leafy vegetables)	**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**	-
Content of active substance (g/kg or g/L)	750 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 610 06 F)	**Residues calculated as:**	Iprodione (I^2) RP 30228 (II^3)and RP 32490 (III^4) both as parent equiv., RP 32596 (IV^5), M610F007 (VI^6)

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 per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH) at Last Treat.**	**Portion Analysed**	**Residues (mg/kg)**					**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I^2**	**II^3**	**III^4**	**IV^5**	**VI^6**		
384189	VL0483	1. 04.06.2010	spray	0.15	500	0.750	3	35-37	wh. plant*	24.80	0.04	0.05	<0.01	-	0	Method
2011/1120988	Danyelle	2. -		0.15	500	0.750	08.07.2010		head	2.65	<0.01	0.08	<0.01	<0.01	14	L0180/01
Stratton Audley/Bicester		3. 29.07.2010		0.15	500	0.750			head	0.48	0.01	0.04	<0.01	<0.01	21	LOQ
United Kingdom									head	0.07	<0.01	0.01	<0.01	<0.01	27	0.01 mg/kg
L100248																

0) actual application rates varied by 10% at most
1) days after last application
2) parent BAS 610 F
3) RP 30228 (Reg. No. 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 32490 (Reg. No. 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
5) RP 32596 (Reg. No. 85831)
6) M610F007 (Reg. No. 5916256)
* without roots

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Lettuce (group 6.3.11 Leafy vegetables)	**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**	-
Content of active substance (g/kg or g/L)	750 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 610 06 F)	**Residues calculated as:**	Iprodione (I^2) RP 30228 (II^3)and RP 32490 (III^4) both as parent equiv., RP 32596 (IV^5), RP 37176 (V^6) and M610F007 (VI^7) as metabolites

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 per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH) at Last Treat.**	**Portion Analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I^2**	**II^3**	**III^4**	**IV^5**	**V^6**	**VI^7**		
384190	VL0483	1. 28.07.2011	spray	0.15	500	0.750	3	35	wh. plant*	14	0.12	0.055	0.01	<0.01	-	0	Method
2012/1273240	Pristalion	2. -		0.15	500	0.750	01.09.2011		head**	0.24	0.023	0.027	<0.01	<0.01	<0.01	14	L0180/01
79356 Eichstetten Germany		3. 20.09. -05.10.2011		0.15	500	0.750			head**	0.052	<0.01	0.012	<0.01	<0.01	<0.01	21	LOQ
L110447									head**	0.015	<0.01	<0.01	<0.01	<0.01	<0.01	27	0.01 mg/kg
384190	VL0483	1. 18.08.2011	spray	0.15	500	0.750	3	43	wh. plant*	17	0.037	0.025	<0.01	<0.01	-	0	Method
2012/1273240	Caipira	2. -		0.15	500	0.750	16.09.2011		head**	0.49	0.023	0.035	<0.01	<0.01	<0.01	13	L0180/01
67203 Oberschaeffolsheim France		3. 05.10.-12.10.2011		0.15	500	0.750			head**	0.079	<0.01	0.01	<0.01	<0.01	<0.01	20	LOQ
L110448									head**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	27	0.01 mg/kg
384190	VL0483	1. 22.07.2011	spray	0.15	500	0.750	3	45	wh. plant*	14	0.028	0.033	<0.01	<0.01	-	0	Method
2012/1273240	Lollo Rosso	2. -		0.15	500	0.750	29.08.2011		head**	0.61	0.037	0.048	<0.01	<0.01	<0.01	13	L0180/01
5853 AB Siebengewald The Netherlands		3. 26.09.2011		0.15	500	0.750			head**	0.058	0.01	0.013	<0.01	<0.01	<0.01	21	LOQ
L110449									head**	0.019	<0.01	<0.01	<0.01	<0.01	<0.01	28	0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Lettuce (group 6.3.11 Leafy vegetables)	**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**	-
Content of active substance (g/kg or g/L)	750 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 610 06 F)	**Residues calculated as:**	Iprodione (I^2) RP 30228 (II^3)and RP 32490 (III^4) both as parent equiv., RP 32596 (IV^5), RP 37176 (V^6) and M610F007 (VI^7) as metabolites

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 per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH) at Last Treat.**	**Portion Analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I^2**	**II^3**	**III^4**	**IV^5**	**V^6**	**VI^7**		
384190	VL0483	1. 14.09.2011	spray	0.15	500	0.750	3	33	wh. plant*	22	0.061	052	<0.01	<0.01	-	0	Method
2012/1273240	Bergamo	2. -		0.15	500	0.750	28.10.2011		head**	3.2	0.13	0.058	0.020	<0.01	<0.01	13	L0180/01
OK279AS Stratton Audley United Kingdom L110450		3. 18.11.2011		0.15	500	0.750			head**	1.6	0.079	0.074	0.014	<0.01	<0.01	21	LOQ
									head**	1.4	0.063	0.064	0.011	<0.01	<0.01	28	0.01 mg/kg

0) actual application rates varied by 10% at most
1) days after last application
2) parent BAS 610 F
3) RP 30228 (Reg. No. 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 32490 (Reg. No. 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
5) RP 32596 (Reg. No. 85831)
6) RP 37176 (Reg. No. 5079612)
7) M610F007 (Reg. No. 5916256)

* without roots ** open leaf varieties

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Lettuce (group 6.3.11 Leafy vegetables)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Greenhouse
Country	Germany	**Other active substance in the formulation**	-
Content of active substance (g/kg or g/L)	500 g/L	**(common name and content)**	
Formulation (e.g. WP)	SC (EXP01862F)	**Residues calculated as:**	Iprodione (BAS 610)

1	2	3	4	5			6	7	8	9	10	11
Report-No. Location (Trial No.)	**Commodity/ Variety**	**1. Sowing/Planting 2. Flowering 3. Harvest**	**Method of Treatment**	**Application Rate per Treatment**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Portion Analysed**	**Residues (mg/kg)**	**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I[3]**		
CA 6.3.2/1 C012437 31290 Villefranche de Lauragais France 00527TL1	Sensai,	1. 02.02.2000 2. - 3. 28.03.2000	spray	118 118 118	629 629 629	0.740 0.740 0.740	3 13.03.2000	118	leaf	2.5	15	Method No AR144-97 LOQ 0.02 mg/kg
CA 6.3.2/1 C012437 35850 Romille France 00527OR1	Omega	1. 20.01.2000 2. - 3. 05.04.2000	spray	147 147 147	501 501 501	0.737 0.737 0.737	3 22.03.2000	43-45	leaf	8.1	14	Method No AR144-97 LOQ 0.02 mg/kg

1) days after last application
2) at last treatment
3) parent BAS 610 F

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Lettuce (group 6.3.11 Leafy vegetables)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Greenhouse
Country	Germany	**Other active substance in the formulation**	-
Content of active substance (g/kg or g/L)	750 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 610 06 F)	**Residues calculated as:**	Iprodione (I^2) RP 30228 (II^3)and RP 32490 (III^4) both as parent equiv., RP 32596 (IV^5), RP 37176 (V^6) and M610F007 (VI^7) as metabolites

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 per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH) at Last Treat.**	**Portion Analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I^2**	**II^3**	**III^4**	**IV^5**	**V^6**	**VI^7**		
384191	VL 0482	1. 24.09.2013	spray	0.15	500	0.750	3	47	head**	17	0.33	0.046	<0.01	<0.01	<0.01	0	Method
2014/1094138	Kinshasa RZ	2. -		0.15	500	0.750	06.11.2013		head**	11	0.16	0.042	<0.01	<0.01	<0.01	7	L0180/01
13870 Rognonas		3. 20.11.2013		0.15	500	0.750			head**	7.8	0.16	0.034	<0.01	<0.01	<0.01	14	LOQ
France									head**	7.7	0.13	0.033	<0.01	<0.01	<0.01	20	0.01 mg/kg
L130587																	
384191	VL 0482	1. 23.08.2013	spray	0.15	500	0.750	3	19/31	head**	24	0.14	0.049	<0.01	0.01	<0.01	0	Method
2014/1094138	Manchester	2. -		0.15	500	0.750	25.09.2013		head**	1.5	0.13	0.107	<0.01	<0.01	<0.01	8	L0180/01
57007 Chalkidona		3. 09.10.2013		0.15	500	0.750			head**	0.53	0.069	0.071	<0.01	<0.01	<0.01	14	LOQ
Greece									head**	0.043	0.049	0.017	<0.01	<0.01	<0.01	21	0.01 mg/kg
L130588																	
384191	VL 4357	1. 02.05.2013	spray	0.15	500	0.750	3	43	head**	0.19	<0.01	<0.01	<0.01	<0.01	<0.01	0	Method
2014/1094138	Concorde RZ	2. -		0.15	500	0.750	01.06.2013		head**	24	0.11	0.063	<0.01	<0.01	<0.01	6	L0180/01
20060 Bellinzago Lomb.		3. 14.06.2013		0.15	500	0.750			head**	14	0.14	0.083	<0.01	<0.01	<0.01	13	LOQ
Italy									head**	5.3	0.099	0.055	<0.01	<0.01	<0.01	19	0.01 mg/kg
L130589																	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Lettuce (group 6.3.11 Leafy vegetables)	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Greenhouse
Country	Germany	**Other active substance in the formulation**	-
Content of active substance (g/kg or g/L)	750 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 610 06 F)	**Residues calculated as:**	Iprodione (I[2]) RP 30228 (II[3])and RP 32490 (III[4]) both as parent equiv., RP 32596 (IV[5]), RP 37176 (V[6]) and M610F007 (VI[7]) as metabolites

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 per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH) at Last Treat.**	**Portion Analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I[2]**	**II[3]**	**III[4]**	**IV[5]**	**V[6]**	**VI[7]**		
384191	VL 0482	1. 06.09.2013	spray	0.15	500	0.750	3	45	head**	36	0.18	0.041	<0.01	0.012	<0.01	0	Method
2014/1094138	Paradai RZ	2. -		0.15	500	0.750	29.10.2013		head**	22	0.11	0.042	<0.01	<0.01	<0.01	6	L0180/01
41710 Utrera, Spain		3. 12.11.2013		0.15	500	0.750			head**	15	0.070	0.054	<0.01	<0.01	<0.01	14	LOQ
L130590									head**	10	0.062	0.055	<0.01	<0.01	<0.01	20	0.01 mg/kg

1) days after last application
2) parent BAS 610 F
3) RP 30228 (Reg. No. 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 32490 (Reg. No. 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
5) RP 32596 (Reg. No. 85831)
6) RP 37176 (Reg. No. 5079612)
7) M610F007 (Reg. No. 5916256)

** open leaf varieties

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Lettuce (group 6.3.11 Leafy vegetables)	**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**	-
Content of active substance (g/kg or g/L)	500 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (Formulation EXP10370A)	**Residues calculated as:**	Iprodione (BAS 610)

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 per Treatment**			**No of Treatm. and Last Date**	**Growth Stage (BBCH)[2]**	**Portion Analysed**	**Residues (mg/kg)**	**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I[3]**		
CA 6.3.2/4 (96-723) C027043 Bologna Italy 96-723	Cappuccio	1. 29.07.1996 2. - 3. 30.09.1996	spray		1000 1000 1000	0.750 0.750 0.750	3 09.06.1996	45	leaf	0.22	21	Method No AR144-97 (modified) LOQ 0.02 mg/kg

0) actual application rates varied by 10% at most
1) days after last application
2) at last treatment
3) parent BAS 610 F

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Lettuce (group 6.3.11 Leafy vegetables)	**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**	-
Content of active substance (g/kg or g/L)	750 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 610 06 F)	**Residues calculated as:**	Iprodione (I^2) RP 30228 (II^3)and RP 32490 (III^4) both as parent equiv., RP 32596 (IV^5), RP 37176 (V^6) and M610F007 (VI^7) as metabolites

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 per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH) at Last Treat.**	**Portion Analysed**	**Residues (mg/kg)**					**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I[3]**	**II[4]**	**III[5]**	**IV[6]**	**VI[7]**		
384189	VL0483	1. 22.09.2010	spray	0.15	500	0.750	3	33-35	wh. plant*	24.80	0.09	0.11	<0.01	-	0	Method
2011/1120988	Simson	2. -		0.15	500	0.750	02.11.2010		head	0.64	0.06	0.09	<0.01	<0.01	14	L0180/01
57007 Thessaloniki		3. 20.11.2010		0.15	500	0.750			head	0.24	0.03	0.06	<0.01	<0.01	21	LOQ
Greece									head	0.06	0.01	0.02	<0.01	<0.01	28	0.01 mg/kg
L100249																
384189	VL0483	1. 30.08.2010	spray	0.15	500	0.750	3	33	wh. plant*	12.98	0.03	0.02	<0.01	-	0	Method
2011/1120988	Lorca	2. -		0.15	500	0.750	15.10.2010		head	0.58	0.02	0.05	<0.01	<0.01	14	L0180/01
14500 Cordoba		3. 13.11.2010		0.15	500	0.750			head	0.23	0.02	0.05	<0.01	<0.01	21	LOQ
Spain									head	0.03	<0.01	<0.01	<0.01	<0.01	27	0.01 mg/kg
L100250																
384189	VL0483	1. 10.09.2010	spray	0.15	500	0.750	3	45	wh. plant*	13.56	0.02	<0.01	<0.01	-	0	Method
2011/1120988	Gentilina	2. -		0.15	500	0.750	13.10.2010		head	0.64	0.01	0.03	<0.01	<0.01	13	L0180/01
14100 Asti, Italy	Impulsion	3. 20.10.2010		0.15	500	0.750			head	0.06	<0.01	<0.01	<0.01	<0.01	21	LOQ
L100251									head	0.05	<0.01	<0.01	<0.01	<0.01	28	0.01 mg/kg
384189	VL0483	1. 04.05.2010	spray	0.15	500	0.750	3	45	wh. plant*	2.18	<0.01	<0.01	<0.01	-	0	Method
2011/1120988	Iris	2. -		0.15	500	0.750	02.06.2010		head	0.01	<0.01	<0.01	<0.01	<0.01	14	L0180/01
34130 Mauguio, France		3. 23.06.2010		0.15	500	0.750			head	0.02	<0.01	<0.01	<0.01	<0.01	21	LOQ
L100252									head	<0.01	<0.01	<0.01	<0.01	<0.01	28	0.01 mg/kg

0) actual application rates varied by 10% at most
1) days after last application
2) parent BAS 610 F
3) RP 30228 (Reg. No. 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 32490 (Reg. No. 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
5) RP 32596 (Reg. No. 85831)
6) M610F007 (Reg. No. 5916256)
* without roots

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Lettuce (group 6.3.11 Leafy vegetables)	**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**	-
Content of active substance (g/kg or g/L)	750 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 610 06 F)	**Residues calculated as:**	Iprodione (I^2) RP 30228 (II^3)and RP 32490 (III^4) both as parent equiv., RP 32596 (IV^5), RP 37176 (V^6) and M610F007 (VI^7) as metabolites

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 per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH) at Last Treat.**	**Portion Analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I^2**	**II^3**	**III^4**	**IV^5**	**V^6**	**VI^7**		
384190	VL0483	1. 08.09.2011	spray	0.15	500	0.750	3	33	wh. plant*	31	0.083	0.083	<0.01	<0.01	-	0	Method
2012/1273240	Manchester	2. -		0.15	500	0.750	06.10.2011		head**	1.0	0.024	0.039	<0.01	<0.01	<0.01	14	L0180/01
59100 Agia Marina		3. 25.10. -05.11.2011		0.15	500	0.750			head**	0.30	<0.01	0.022	<0.01	<0.01	<0.01	21	LOQ
Greece									head**	0.076	<0.01	0.012	<0.01	<0.01	<0.01	28	0.01 mg/kg
L110451																	
384190	VL0483	1. 18.08.2011	spray	0.15	500	0.750	3	41-42	wh. plant*	19	078	0.044	<0.01	<0.01	-	0	Method
2012/1273240	Caipira	2. -		0.15	500	0.750	16.09.2011		head**	0.072	0.017	0.020	<0.01	<0.01	<0.01	14	L0180/01
17457 Riudellots de la Selva		3. 06.-15.10.2011		0.15	500	0.750			head**	0.037	<0.01	0.012	<0.01	<0.01	<0.01	20	LOQ
Spain									head**	<0.01	<0.01	0.051	<0.01	<0.01	<0.01	28	0.01 mg/kg
L110452																	
384190	VL0483	1. 26.07.2011	spray	0.15	500	0.750	3	39	wh. plant*	17	0.021	0.01	<0.01	<0.01	-	0	Method
2012/1273240	Foglia Di	2. -		0.15	500	0.750	19.08.2011		head**	0.18	0.047	0.03	<0.01	<0.01	<0.01	15	L0180/01
15072 Casalcermelli	Querica	3. 09.-18.09.2011		0.15	500	0.750			head**	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	21	LOQ
Italy									head**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	27	0.01 mg/kg
L110453																	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Iprodione	**Commercial Product (name)**	-
Crop/crop group:	Lettuce (group 6.3.11 Leafy vegetables)	**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**	-
Content of active substance (g/kg or g/L)	750 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 610 06 F)	**Residues calculated as:**	Iprodione (I^2) RP 30228 (II^3)and RP 32490 (III^4) both as parent equiv., RP 32596 (IV^5), RP 37176 (V^6) and M610F007 (VI^7) as metabolites

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 per Treatment[0]**			**No of Treatm. and Last Date**	**Growth Stage (BBCH) at Last Treat.**	**Portion Analysed**	**Residues (mg/kg)**						**DALA[1] (days)**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**I^2**	**II^3**	**III^4**	**IV^5**	**V^6**	**VI^7**		
384190	VL0483	1. 08.08.2011	spray	0.15	500	0.750	3	43	wh. plant*	20	0.055	0.029	<0.01	<0.01	-	0	Method
2012/1273240	Batavia	2. -		0.15	500	0.750	30.08.2011		head**	0.17	0.022	0.030	<0.01	<0.01	<0.01	14	L0180/01
31790 Saint Jory, France		3. 19.-20.09.2011		0.15	500	0.750			head**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	21	LOQ
L110454									head**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	28	0.01 mg/kg

0) actual application rates varied by 10% at most
1) days after last application
2) parent BAS 610 F
3) RP 30228 (Reg. No. 5079647), conversion factor from metabolite to parent BAS 610 F is 1.0
4) RP 32490 (Reg. No. 5079628), conversion factor from metabolite to parent BAS 610 F is 1.146
5) RP 32596 (Reg. No. 85831),
6) RP 37176 (Reg. No. 5079612)
7) M610F007 (Reg. No. 5916256)

* without roots ** open leaf varieties

BAS 610F

DOCUMENT M-CA, Section 7

FATE AND BEHAVIOUR IN THE ENVIRONMENT

Compiled by:

[redacted]

[redacted]

Tel.No.:
Fax.No.:
E-mail: [redacted]

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

A concordance list of structures and designations of reference compounds used during environmental fate studies is given below.

The already peer-reviewed studies as well as the new studies were performed using uniformly aniline-ring ^{14}C-labelled iprodione.

Table 7-1: Substances and metabolites; structures, codes, synonyms

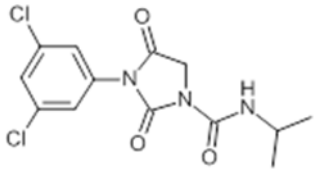
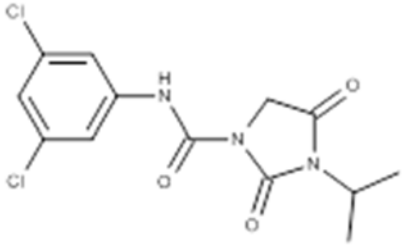
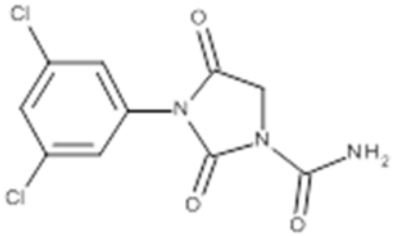
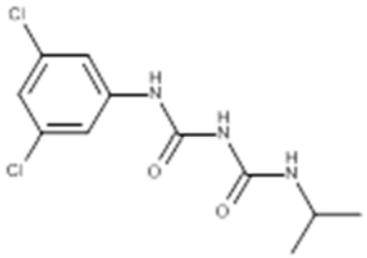
Code Number [1)] (Synonyms)	Description	Compound found in:	Structure
Iprodione BAS 610 F RP 26019 M610F000 Reg.No. 101169 (MW 330.17)	3-(3,5-dichlorophenyl)-N-isopropyl-2,4-dioxoimidazolidine-1-carboxamide	soil water sediment	
RP 30228 M610F001 Reg.No. 5079647 (MW 330.17)	N-(3,5-dichlorophenyl)-2,4-dioxo-3-isopropylimidazolidine-1-carboxamide	soil water sediment	
RP 32490 Reg.No. 5079628 (MW 288.09)	3-(3,5-dichlorophenyl)-2,4-dioxoimidazolidine-1-carboxamide	(water)	
RP 36221 M610F002 Reg.No. 5079618 (MW 290.15)	1-(3,5-dichlorophenyl)-5-isopropyl biuret	soil	

Table 7-1: Substances and metabolites; structures, codes, synonyms

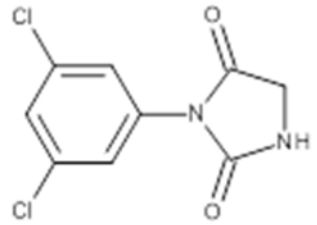
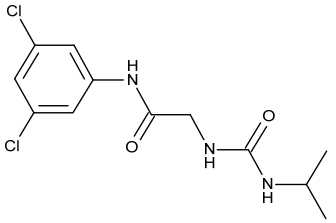
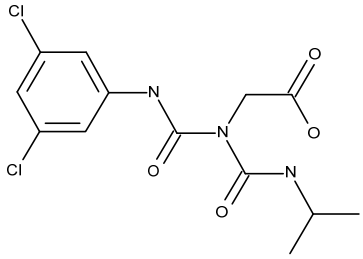
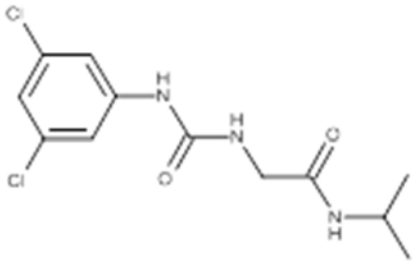
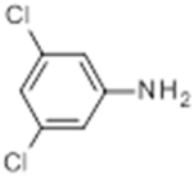
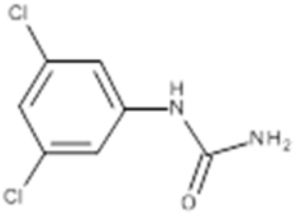
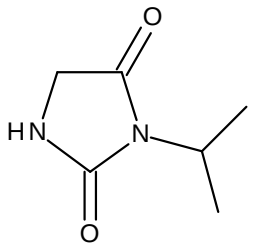
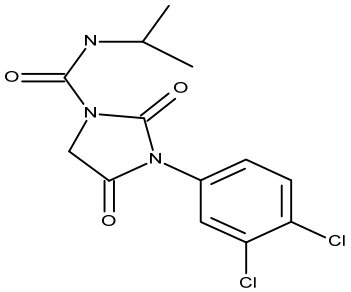
Code Number [1)] (Synonyms)	Description	Compound found in:	Structure
RP 25040 M610F004 Reg.No. 207099 (MW 245.1)	3-(3,5-dichlorophenyl)-2,4-dioxoimidazolidine	soil	
RP 37176 Reg.No. 5079612 (MW 304.2)	N-(3,5-dichlorophenyl)-2-(isopropylcarbamoylamino)acetamide	Hydrolysis (higher temp.)	
M610F014 RP 35606 Reg.No. 5079626 (MW 348.2)	2-[(3,5-dichlorophenyl)carbamoyl-(isopropylcarbamoyl)amino]acetic acid	Soil water sediment	
RP 36233 Reg.No. 5079632 (MW 304.18)	N-isopropyl-[3-(3,5-dichlorophenyl)ureido]acetamide	hydrolysis (higher temp.)	
RP 32596, 3,5-DCA M610F012 Reg.No. 85831 (MW 162.02)	3,5-dichloroaniline	Soil (water)	
RP 44247 M610F006 Reg.No. 89517 LS720942 (MW 205.0)	(3,5-dichlorophenyl)urea	Soil (photolysis)	

Table 7-1: Substances and metabolites; structures, codes, synonyms

Code Number [1)] (Synonyms)	Description	Compound found in:	Structure
RP 30181 Reg. No.2079634	3-isopropylimidazolidine-2,4-dione	Proposed Literature	
RP 40837	3-(3,4-dichlorophenyl)-N-isopropyl-2,4-dioxo-imidazolidine-1-carboxamide	Aqueous photolysis	

1) For RP codes, different formats are in use:
"RP xxxxx" or or "RPxxxxx" or "RP0xxxxx"
Independent from these format differences, the last 5 digit "xxxxx" are unique for every compound

CA 7.1 Fate and behaviour in soil

CA 7.1.1 Route of degradation in soil

Studies presented in the original Annex II Dossier (1995):
Many studies were performed with iprodione during its registration history. An overview is given in Table 7.1.1-1. Except for the study of Waring [*Waring (1993) – R014527*] they were no longer considered as relevant, due to high overdosing, insufficient duration or no longer state of the art identification technique. The study of Waring is summarized in this supplementary dossier to provide a complete view on the fate of iprodione in the environment.

Table 7.1.1-1: List of degradation studies in soil performed with iprodione

Reference	Soil	Application rate [mg/kg]	Incubation temperature	Incubation period [days]	Remark
Buys et al, 1974	Brie soil Compost	25 50	Room temp Room temp.	54 54	Not considered relevant
Gouot et al., 1976	Loamy	2 5	23-25 °C 23-25 °C	387 382	Not considered relevant
Gouot et al., 1977	Clay loam Silty clay	1 10 10 10	25°C 25°C 15°C 25°C	385 385 385 385	Not considered relevant
Spare and Po Yung Lu 1978	Loam Sterilised loam Sandy loam Sterilised sandy loam	40 40 40 40	25°C 25°C 25°C 25°C	30 30 30 30	Not considered relevant
Waring 1993 (R014527)	Sandy loam	10	25°C	276	Summarised

Submission of not yet peer-reviewed studies in this AIR3-Dossier:
A new study was performed in four soils [*Hartman et al. (2014a) – 2013/1240319, CA 7.1.2.1.1/1*] which provides most reliable results for the aerobic degradation of iprodione in soil.

CA 7.1.1.1 Aerobic degradation

Report:	CA 7.1.1.1/1 Hartman M. et al., 2014a Aerobic soil metabolism and rate of degradation of BAS 610 F (Iprodione) in EU soils 2013/1240319
Guidelines:	OECD 307, EPA 835.4100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany) The draft report is referred as *Bayer 2013* in some documents which were finalised in parallel to this report.

Executive Summary

The objective of this study was to determine the route and rate of degradation of BAS 610 F-iprodione in four aerobic soils at a temperature of 20°C.

The aerobic soil degradation of iprodione was conducted using four typical agricultural soils from Germany, which were freshly collected from the field and passed through a 2 mm sieve before use. The soils were treated in a batch application with [phenyl-U-^{14}C]-iprodione at a nominal rate of 5.3 mg a.s. /kg (equivalent to ca. 2000 g a.s. /ha assuming a soil layer of 2.5 cm and bulk density of 1.5 g/cm^3). The treated soils were incubated at 40% maximum water holding capacity (MWHC) at 20 ± 2°C in the dark during 120 days. A closed incubation system with continuous aeration (moistened air) was used with an attached trapping system for the determination of volatile compounds.

The material balance ranged from 91.0% to 103.0% total applied radioactivity (TAR) for the four soils and all sampling times, with average values of 97.7 - 98.4% TAR throughout the incubation period of 120 days.

Iprodione was degraded in all the tested soils. Several metabolites were detected in the soil extracts and identified as M610F004 (Reg. No. 207099, RP 25040), M610F012 (Reg. No. 85831, RP 32596), M610F014 (Reg. No. 5079626, RP 35606), M610F002 (Reg. No. 5079618, RP 36221) and M610F001 (Reg. No. 5079647, RP 30228). Except in the soil LUFA 2.2, these metabolites reached concentration above 5% TAR. Other minor degradation products (<5% TAR) were also detected, each of them representing 0.9% - 1.4% TAR. Only low formation of $^{14}CO_2$ and volatile ^{14}C-organic compounds was observed in all four soils, with CO_2 amounts averaged over the two replicates reaching in total 0.6 - 1.7% TAR after 120 days of incubation.

The soil degradation pathway for iprodione under aerobic conditions primary involved the opening of the imidazolidindione ring, to form the carboxylic acid (M610F014), followed by further degradation steps ultimately leading to the formation of CO_2 and incorporation of the transformation products into bound residues.

The DT_{50} values for iprodione in soil LUFA 2.2, Li10, LUFA 2.3 and LUFA 5M were 91.1, 43.5, 9.6 and 6.3 days, respectively. The DT_{90} values ranged from 73.7 days to 376.9 days.

Non-extractable radioactive residues (NER) in the four soils were formed in substantial amounts during the course of the study. Characterisation of NER revealed that the major portion of radioactivity was tightly bound to the soil matrix or part of the humic acid fraction. Since iprodione was detectable only in very low amounts in the NER, it is assumed that no significant amounts of iprodione can be released from the bound residues even with harsh and artificial extraction methods.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Internal code:	BAS 610 F
CAS Number.:	36734-19-7
Chemical name (IUPAC):	3-(3,5-dichlorophenyl)-N-isopropyl-2,4-dioxoimidazolidine-1-carboxamide
Molecular weight:	330.1697 g/mol (unlabelled)
Position of radiolabel:	phenyl-U-^{14}C
Specific activity:	6.69 MBq/mg (401400 dpm/µg)
Chemical purity:	94.9%
Radiochemical purity:	95.3%

2. Soils

Soils used in this study four typical agricultural soils from Germany, which were freshly collected from the field and passed through a 2 mm sieve before use. The soil characteristics are summarised in Table 7.1.1.1-1.

Table 7.1.1.1-1: Soil characteristics

Soil designation		LUFA 2.2 (11/736/02)	Li 10 (11/1680/02)	LUFA 2.3 (11/570/02)	LUFA 5M (11/1651/02)
Origin		Hanhofen, RP, Germany	Limburgerhof, RP, Germany	Offenbach, RP, Germany	Mechtersheim, RP, Germany
USDA Particle size distribution [%]					
Clay	<0.002 mm	4.7	4.3	8.3	12.6
Silt	0.002 - 0.050 mm	15.2	12.2	23.1	27.9
Sand	0.050 – 2 mm	80.1	83.5	68.6	59.5
Soil class		Loamy sand	Loamy sand	Sandy loam	Sandy loam
DIN Particle size distribution [%]					
Clay	<0.002 mm	4.7	4.3	8.3	12.6
Silt	0.002 - 0.063 mm	15.9	13.9	24.8	35.5
Sand	0.063 – 2 mm	79.3	81.7	66.9	51.9
Soil class		Silty sand	Silty sand	Loamy sand	Loamy sand
Organic C [%]		1.53	0.95	0.99	0.95
Organic matter [%]*		2.64	1.64	1.71	1.64
pH ($CaCl_2$)		5.5	6.2	6.7	7.3
pH (H_2O)		6.2	6.9	7.4	8.1
Cation exchange capacity [$cmol^+/kg$]		6.6	5.5	7.5	10.2
Maximum water holding capacity [g/100g dry soil]		33.4	23.2	28.2	29.4
Microbial biomass [mg C/100g dry soil]		37.8	23.6	27.8	26.1

RP= Rhineland-Palatinate
*calculated: organic matter = organic C x 1.724

B. STUDY DESIGN

1. Experimental conditions

The test substance was applied at a nominal concentration of 5.3 mg ^{14}C-labelled iprodione /kg dry soil (equivalent to ca. 2000 g a.s. /ha assuming a soil layer of 2.5 cm and bulk density of 1.5 g/cm^3).

Soil aliquots of 50 g (dry weight basis) were weighed into test vessels and placed in an incubation cabinet. The treated soils were incubated at 40% maximum water holding capacity (MWHC) at 20 ± 2°C in the dark during 120 days. Throughout the incubation period the samples were continuously aerated with a slight stream of moistened and CO_2 free air. Each series of vessels ended up in a trapping system for trapping CO_2 and volatiles consisting of a sequence of three gas washing flasks: one with 0.5M NaOH, one with ethylene glycol and another with 0.5M H_2SO_4.

2. Sampling

Duplicate soil samples were taken at 3, 7, 14, 23-27, 41-43, 57-59, 91-92, and 120 days after treatment (DAT).

The liquid traps were assayed at all sampling times (except time-0) and replaced with fresh solutions.

3. Description of analytical procedures

The soil samples were extracted two to three times with 100 mL of acetonitrile, followed by two to four extractions with 50% of acetonitrile in water (100-200 mL each) and then by two to four extractions with 75% of acetonitrile in water (100-200 mL each). For each extraction step, the samples were shaken at 300 rpm for about 20 minutes at room temperature, except for the last two extractions of the samples at 91 DAT and 120 DAT, which were shaken overnight (Li 10, LUFA 5M and LUFA 2.3 soils). Afterwards the individual extracts were analysed by liquid scintillation counting (LSC). The combined acetonitrile extracts and the combined acetonitrile/water extracts were concentrated and analysed by radio-HPLC. The remaining soil, after extraction, was combusted in order to determine the amount of non-extractable soil radioactive residues (NER, bound residues). A full material balance was provided for each sampling interval.

Bound residues characterisation was also performed with selected samples from all four soils by extraction with 0.5N NaOH solution (35 mL x 3 times). The extract was separated into humic and fulvic fractions by acid precipitation. The fulvic fraction was characterised by partitioning with ethyl acetate.

Identification of parent and its transformation products was performed by retention matching with reference standards using radio-HPLC and confirmation by LC-MS/MS. Radioactivity in the extracts was analysed (LSC).

The amounts of CO_2 produced were determined at each sampling time (excluding 0 DAT). The traps were assayed directly by adding aliquots of the trapping solutions into liquid scintillation fluid and counting by liquid scintillation counting (LSC).

4. Calculation of the degradation rate

The calculation of the DT_{50} and DT_{90} values for iprodione was performed following the recommendations 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 2.0, 434 pp*]. Degradation products occurring during the study period were not considered in the kinetic evaluation. Optimisation of model parameters, including estimation of parameter standard errors, was performed using the software package KinGUI version 2.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The material mass balance from the soils treated with ^{14}C-labelled iprodione is summarised in Table 7.1.1.1-2 to Table 7.1.1.1-5. The material mass balance ranged from 91.1% to 103.0% TAR for all soils and sampling times, with average values of 97.7 - 98.4% TAR throughout the incubation period of 120 days.

B. EXTRACTABLE AND BOUND RESIDUES

The distribution of radioactivity in extractable residues (EER), non-extractable residues (NER, bound residues) and volatiles for each soil is shown in Table 7.1.1.1-2 to Table 7.1.1.1-5.

The amount of extractable radioactivity (ERR) in the tested soils continuously decreased from an average of 98.5 - 99.3% TAR at day 0 to an average of 29.4 - 58.0% TAR at 120 days. NER increased from an average of 0.8 - 1.5 % TAR immediately after treatment to 40.4 - 61.2% TAR after 120 DAT.

Only low formation of $^{14}CO_2$ and volatile ^{14}C-organic compounds was observed in all four soils, with CO_2 amounts averaged over the two replicates reaching in total 0.6 - 1.7% TAR after 120 days.

Table 7.1.1.1-2: Distribution of the radioactivity in LUFA 2.2 soil after treatment with ^{14}C-iprodione under aerobic conditions [% TAR]

DAT	ERR			NER	Volatiles *				Material balance
	Acetonitrile	Acetonitrile + water	Total		CO_2	Ethylene glycol trap	H_2SO_4 trap	Total	
0 (Rep 1)	98.3	1.7	100.0	0.8	n.a	n.a	n.a	n.a	100.8
0 (Rep 2)	96.4	2.1	98.5	0.7	n.a	n.a	n.a	n.a	99.2
0 (mean)	**97.3**	**1.9**	**99.2**	**0.8**	**n.a**	**n.a**	**n.a**	**n.a**	**100.0**
3 (Rep 1)	94.7	4.0	98.7	2.1	0.1	0.0	0.0	0.1	100.9
3 (Rep 2)	92.7	3.8	96.5	2.0	0.1	0.0	0.0	0.1	98.6
3 (mean)	**93.7**	**3.9**	**97.6**	**2.1**	**0.1**	**0.0**	**0.0**	**0.1**	**99.8**
7 (Rep 1)	92.3	4.4	96.7	3.9	0.1	0.0	0.0	0.1	100.7
7 (Rep 2)	93.5	4.4	98.0	3.6	0.2	0.0	0.0	0.2	101.8
7 (mean)	**92.9**	**4.4**	**97.3**	**3.7**	**0.2**	**0.0**	**0.0**	**0.2**	**101.2**
14 (Rep 1)	87.8	4.7	92.4	7.6	0.2	0.0	0.0	0.2	100.2
14 (Rep 2)	84.2	4.3	88.5	7.4	0.3	0.0	0.0	0.3	96.2
14 (mean)	**86.0**	**4.5**	**90.5**	**7.5**	**0.2**	**0.0**	**0.0**	**0.2**	**98.2**
27 (Rep 1)	79.8	4.3	84.0	13.5	0.3	0.0	0.0	0.3	97.8
27 (Rep 2)	79.4	4.3	83.6	13.6	0.3	0.0	0.0	0.4	97.6
27 (mean)	**79.6**	**4.3**	**83.8**	**13.5**	**0.3**	**0.0**	**0.0**	**0.3**	**97.7**
41 (Rep 1)	74.7	5.0	79.6	18.0	0.3	0.1	0.0	0.4	98.0
41 (Rep 2)	74.5	4.7	79.3	19.7	0.4	0.0	0.0	0.5	99.5
41 (mean)	**74.6**	**4.9**	**79.4**	**18.9**	**0.4**	**0.1**	**0.0**	**0.4**	**98.7**
58 (Rep 1)	63.0	4.6	67.6	24.1	0.3	0.2	0.0	0.5	92.2
58 (Rep 2)	60.1	4.7	64.7	24.5	0.5	0.1	0.0	0.6	89.8
58 (mean)	**61.5**	**4.6**	**66.1**	**24.3**	**0.4**	**0.1**	**0.0**	**0.6**	**91.0**
92 (Rep 1)	54.8	7.3	62.2	33.2	0.4	0.3	0.1	0.7	96.1
92 (Rep 2)	54.9	7.1	62.0	32.1	0.5	0.1	0.2	0.8	94.9
92 (mean)	**54.9**	**7.2**	**62.1**	**32.7**	**0.4**	**0.2**	**0.1**	**0.7**	**95.5**
120 (Rep 1)	51.2	7.3	58.5	41.4	0.6	0.3	0.1	1.0	100.9
120 (Rep 2)	50.3	7.2	57.4	39.5	0.6	0.1	0.2	0.9	97.8
120 (mean)	**50.7**	**7.2**	**58.0**	**40.4**	**0.6**	**0.2**	**0.1**	**1.0**	**99.4**

TAR = total applied radioactivity (100% = 5.192 mg/kg) **total average: 97.9**
DAT = days after treatment
ERR = extractable radioactive residues
NER = non-extractable radioactive residues
n.a. = not applicable
Rep = replicate
* = cumulative values

Table 7.1.1.1-3: Distribution of the radioactivity in Li 10 soil after treatment with ^{14}C-iprodione under aerobic conditions [% TAR]

DAT	ERR			NER	Volatiles *				Material balance
	Acetonitrile	Acetonitrile + water	Total		CO_2	Ethylene glycol trap	H_2SO_4 trap	Total	
0 (Rep 1)	96.3	2.0	98.3	0.7	n.a	n.a	n.a	n.a	99.0
0 (Rep 2)	97.8	2.5	100.3	0.7	n.a	n.a	n.a	n.a	101.0
0 (mean)	**97.1**	**2.3**	**99.3**	**0.7**	**n.a**	**n.a**	**n.a**	**n.a**	**100.0**
3 (Rep 1)	**91.8**	**6.4**	**98.2**	**2.1**	**0.1**	**0.0**	**0.0**	**0.1**	**100.4**
7 (Rep 1)	**87.6**	**7.1**	**94.7**	**4.7**	**0.1**	**0.0**	**0.0**	**0.1**	**99.5**
14 (Rep 1)	**86.9**	**7.0**	**93.9**	**8.2**	**0.2**	**0.0**	**0.0**	**0.2**	**102.3**
24 (Rep 1)	**79.3**	**6.6**	**86.0**	**14.7**	**0.3**	**0.0**	**0.0**	**0.3**	**101.0**
42 (Rep 2)	**68.0**	**5.9**	**73.9**	**17.3**	**0.3**	**0.4**	**0.0**	**0.8**	**92.0**
59 (Rep 1)	53.6	7.2	60.8	39.0	0.4	0.2	0.1	0.8	100.6
59 (Rep 2)	60.7	8.7	69.4	28.0	0.4	0.7	0.1	1.2	98.6
59 (mean)	**57.2**	**7.9**	**65.1**	**33.5**	**0.4**	**0.4**	**0.1**	**1.0**	**99.6**
91 (Rep 1)	**39.4**	**12.9**	**52.3**	**42.4**	**0.7**	**0.4**	**0.1**	**1.2**	**95.9**
120 (Rep 1)	20.8	14.6	35.4	53.2	1.1	0.6	0.1	1.8	90.4
120 (Rep 2)	24.7	14.6	39.4	57.6	0.7	1.1	1.2	3.0	100.0
120 (mean)	**22.8**	**14.6**	**37.4**	**55.4**	**0.9**	**0.8**	**0.7**	**2.4**	**95.2**

TAR = total applied radioactivity (100% = 5.176 mg/kg) **total average: 98.4**
DAT = days after treatment
ERR = extractable radioactive residues
NER = non-extractable radioactive residues
n.a. = not applicable
Rep = replicate
* = cumulative values

Table 7.1.1.1-4: Distribution of the radioactivity in LUFA 2.3 soil after treatment with ^{14}C-iprodione under aerobic conditions [% TAR]

DAT	ERR			NER	Volatiles *				Material balance
	Acetonitrile	Acetonitrile + water	Total		CO_2	Ethylene glycol trap	H_2SO_4 trap	Total	
0 (Rep 1)	92.4	4.9	97.3	1.5	n.a	n.a	n.a	n.a	98.8
0 (Rep 2)	94.7	5.0	99.6	1.6	n.a	n.a	n.a	n.a	101.2
0 (mean)	**93.5**	**4.9**	**98.5**	**1.5**	**n.a**	**n.a**	**n.a**	**n.a**	**100.0**
3 (Rep 1)	**76.3**	**16.5**	**92.8**	**7.5**	**0.1**	**0.0**	**0.0**	**0.1**	**100.4**
7 (Rep 1)	**68.0**	**19.1**	**87.1**	**12.5**	**0.2**	**0.0**	**0.0**	**0.2**	**99.8**
14 (Rep 1)	**60.5**	**16.5**	**77.1**	**23.1**	**0.4**	**0.0**	**0.0**	**0.4**	**100.6**
23 (Rep 1)	**49.3**	**13.5**	**62.8**	**34.3**	**0.6**	**0.1**	**0.0**	**0.7**	**97.8**
43 (Rep 1)	**35.6**	**13.4**	**49.0**	**46.8**	**0.9**	**0.2**	**0.0**	**1.1**	**96.8**
57 (Rep 1)	31.5	18.3	49.8	46.7	1.0	0.4	0.0	1.4	97.9
57 (Rep 2)	30.0	18.7	48.7	41.8	0.9	0.6	0.0	1.5	91.9
57 (mean)	**30.7**	**18.5**	**49.3**	**44.2**	**0.9**	**0.5**	**0.0**	**1.4**	**94.9**
91 (Rep 1)	**25.7**	**16.5**	**42.2**	**51.7**	**1.2**	**0.7**	**0.0**	**1.9**	**95.8**
120 (Rep 1)	20.5	15.1	35.6	57.6	1.6	0.8	0.0	2.4	95.6
120 (Rep 2)	19.3	15.3	34.6	56.9	1.8	1.0	0.2	3.0	94.5
120 (mean)	**19.9**	**15.2**	**35.1**	**57.2**	**1.7**	**0.9**	**0.1**	**2.7**	**95.0**

TAR = total applied radioactivity (100% = 5.244 mg/kg) **total average: 97.9**
DAT = days after treatment
ERR = extractable radioactive residues
NER = non-extractable radioactive residues
n.a. = not applicable
Rep = replicate
* = cumulative values

Table 7.1.1.1-5: Distribution of the radioactivity in LUFA 5M soil after treatment with ^{14}C-iprodione under aerobic conditions [% TAR]

DAT	ERR			NER	Volatiles *				Material balance
	Acetonitrile	Acetonitrile + water	Total		CO_2	Ethylene glycol trap	H_2SO_4 trap	Total	
0 (Rep 1)	94.0	5.2	99.2	1.0	n.a	n.a	n.a	n.a	100.1
0 (Rep 2)	94.8	4.1	98.9	1.0	n.a	n.a	n.a	n.a	99.9
0 (mean)	**94.4**	**4.6**	**99.0**	**1.0**	**n.a**	**n.a**	**n.a**	**n.a**	**100.0**
3 (Rep 1)	**69.9**	**28.8**	**98.7**	**4.2**	**0.1**	**0.0**	**0.0**	**0.1**	**103.0**
7 (Rep 1)	**57.3**	**35.5**	**92.8**	**6.5**	**0.2**	**0.0**	**0.0**	**0.2**	**99.6**
14 (Rep 1)	**54.3**	**30.6**	**84.9**	**11.6**	**0.2**	**0.2**	**0.0**	**0.4**	**96.8**
23 (Rep 1)	**49.8**	**28.5**	**78.3**	**19.5**	**0.3**	**0.3**	**0.0**	**0.7**	**98.5**
43 (Rep 1)	**42.7**	**22.5**	**65.2**	**26.3**	**0.4**	**0.7**	**0.2**	**1.3**	**92.8**
57(Rep 1)	38.4	17.9	56.4	37.6	0.6	1.0	0.2	1.8	95.7
57 (Rep 2)	38.5	15.7	54.1	43.1	1.3	0.3	0.0	1.6	98.9
57 (mean)	**38.4**	**16.8**	**55.2**	**40.4**	**0.9**	**0.6**	**0.1**	**1.7**	**97.3**
91 (Rep 1)	**24.6**	**15.1**	**39.7**	**54.2**	**0.8**	**1.8**	**0.5**	**3.0**	**97.0**
120 (Rep 1)	16.1	13.1	29.2	60.8	1.2	1.9	0.5	3.6	93.6
120 (Rep 2)	16.1	13.6	29.7	61.6	2.1	0.9	0.0	3.0	94.3
120 (mean)	**16.1**	**13.3**	**29.4**	**61.2**	**1.6**	**1.4**	**0.3**	**3.3**	**94.0**

TAR = total applied radioactivity (100% = 5.243 mg/kg) **total average: 97.7**
DAT = days after treatment
ERR = extractable radioactive residues
NER = non-extractable radioactive residues
n.a. = not applicable
Rep = replicate
* = cumulative values

Results from bound residues characterisation are presented in Table 7.1.1.1-6 to Table 7.1.1.1-9. Upon extraction with NaOH, roughly one third to one half of the NER from the 4 soils remained un-extractable. This portion of un-extractable residues reached average maximum amounts of 15.1%, 22.1%, 34.2% and 29.1% TAR for the 120 DAT samples of soils LUFA 2.2, Li 10, LUFA 2.3 and LUFA 5M, respectively, and was assigned to the humin fraction. Smaller amounts of radioactivity were associated with the humic acid and the fulvic acid fraction.

For the 120 DAT samples, the humic acid fraction ranged from 8.5% to 18.2% TAR (mean of two replicates) and the fulvic acid fraction ranged from 7.2% to 12.7% TAR (mean of two replicates) in the four soils tested. A major portion of radioactivity could be partitioned from the fulvic acid fraction into ethyl acetate phase (from an averaged value of 4.6% to 7.5% TAR at 120 DAT in the tested soils), the remaining radioactivity in the aqueous phase accounting for only low amounts (1.7% to 3.5% TAR at 120 DAT in the tested soils).

Table 7.1.1.1-6: Characterisation of non-extractable residues in soil LUFA 2.2 treated with ^{14}C-iprodione [% TAR]

Days after treatment	NER	NaOH extract	Humic acids	Fulvic acids (total)	Fulvic acids (partition)		Humins
					Aqueous phase	Organic phase	
14 (rep 1)	7.6	4.7	2.6	1.6	0.4	1.1	2.3
14 (rep 2)	7.4	4.5	2.5	1.6	0.4	1.1	2.0
14 (mean)	**7.5**	**4.6**	**2.5**	**1.6**	**0.4**	**1.1**	**2.2**
27 (rep 1)	13.5	8.4	4.5	2.7	0.7	1.9	3.6
27 (rep 2)	13.6	8.3	4.6	2.7	0.7	1.9	3.5
27 (mean)	**13.5**	**8.3**	**4.5**	**2.7**	**0.7**	**1.9**	**3.6**
41 (rep 1)	18.0	10.7	6.4	3.6	0.9	2.4	6.6
41 (rep 2)	19.7	11.2	6.6	3.9	0.9	2.7	8.3
41 (mean)	**18.9**	**11.0**	**6.5**	**3.7**	**0.9**	**2.6**	**7.4**
58 (rep 1)	24.1	13.8	8.2	4.7	1.1	3.3	8.5
58 (rep 2)	24.5	14.0	8.3	4.9	1.2	3.3	9.2
58 (mean)	**24.3**	**13.9**	**8.3**	**4.8**	**1.1**	**3.3**	**8.9**
92 (rep 1)	33.2	19.8	11.9	6.8	1.6	4.5	12.3
92 (rep 2)	32.1	19.3	11.3	6.8	1.5	4.5	10.8
92 (mean)	**32.7**	**19.5**	**11.6**	**6.8**	**1.5**	**4.5**	**11.6**
120 (rep 1)	41.4	22.9	13.4	7.0	1.7	4.5	16.3
120 (rep 2)	39.5	21.5	14.4	7.3	1.8	4.7	13.9
120 (mean)	**40.4**	**22.2**	**13.9**	**7.2**	**1.7**	**4.6**	**15.1**

NER = non-extractable residues
rep = replicate
TAR = total applied radioactivity

Table 7.1.1.1-7: Characterisation of non-extractable residues in soil Li 10 treated with ^{14}C-iprodione [% TAR]

Days after treatment	NER	NaOH extract	Humic acids	Fulvic acids (total)	Fulvic acids (partition)		Humins
					Aqueous phase	Organic phase	
7 (rep 1)	4.7	3.1	1.6	1.5	0.3	1.1	1.3
14 (rep 1)	8.2	5.3	2.8	2.3	0.5	1.7	2.4
24 (rep 1)	14.7	9.3	5.5	3.6	0.7	2.6	4.8
120 (rep 1)	53.2	29.2	18.1	10.9	3.2	6.5	21.7
120 (rep 2)	57.6	29.5	18.3	10.9	3.0	6.7	22.5
120 (mean)	**55.4**	**29.4**	**18.2**	**10.9**	**3.1**	**6.6**	**22.1**

NER = non-extractable residues
rep = replicate
TAR = total applied radioactivity

Table 7.1.1.1-8: Characterisation of non-extractable residues in soil LUFA 2.3 treated with ^{14}C-iprodione [% TAR]

Days after treatment	NER	NaOH extract	Humic acids	Fulvic acids (total)	Fulvic acids (partition)		Humins
					Aqueous phase	Organic phase	
3 (rep 1)	7.5	5.3	1.5	3.8	0.3	3.4	1.8
7 (rep 1)	12.5	8.0	2.5	5.5	0.4	5.0	4.5
14 (rep 1)	23.1	12.0	4.2	7.7	0.8	6.8	9.4
23 (rep 1)	34.3	14.3	4.9	10.1	1.2	7.8	19.1
120 (rep 1)	57.6	18.6	7.9	11.0	2.7	7.0	32.7
120 (rep 2)	56.9	20.1	9.1	11.2	2.9	7.1	35.7
120 (mean)	**57.2**	**19.4**	**8.5**	**11.1**	**2.8**	**7.0**	**34.2**

NER = non-extractable residues
rep = replicate
TAR = total applied radioactivity

Table 7.1.1.1-9: Characterisation of non-extractable residues in soil LUFA 5M treated with ^{14}C-iprodione [% TAR]

Days after treatment	NER	NaOH extract	Humic acids	Fulvic acids (total)	Fulvic acids (partition)		Humins
					Aqueous phase	Organic phase	
7 (rep 1)	6.5	4.5	1.8	2.5	0.4	2.0	1.9
14 (rep 1)	11.6	7.4	3.4	3.9	0.6	3.1	4.0
23 (rep 1)	19.5	10.0	4.5	5.7	0.9	4.1	8.0
120 (rep 1)	60.8	25.8	14.1	12.8	3.4	7.5	27.6
120 (rep 2)	61.6	24.0	13.9	12.5	3.5	7.5	30.6
120 (mean)	**61.2**	**24.9**	**14.0**	**12.7**	**3.5**	**7.5**	**29.1**

NER = non-extractable residues
rep = replicate
TAR = total applied radioactivity

C. VOLATILISATION

Only low mineralisation was observed in all four soils, with CO_2 amounts averaged over the two replicates reaching in total 0.6% (LUFA 2.2), 0.9% (Li 10), 1.7% (LUFA 2.3) and 1.6% TAR (LUFA 5M) after 120 DAT (Table 7.1.1.1-2 to Table 7.1.1.1-5). Low amounts of volatiles were detected in the ethylene glycol and H_2SO_4 traps, with average amounts never exceeding 1.4% TAR in the ethylene glycol trap and 0.7% TAR in the H_2SO_4 trap.

E. TRANSFORMATION OF PARENT COMPOUND

Radio-HPLC analysis of the soil extracts showed degradation of iprodione in the four tested soils.

Iprodione concentrations decreased from average of 92.5% to 97.3% TAR in all soils to an average of 44.1%, 7.5%, 7.3% and 4.5% in soils LUFA 2.2, Li 10, LUFA 2.3 and LUFA 5 M, respectively. Several metabolites were detected in soil extracts of all soils, with maximum amounts of 3.7%, 12.6%, 13.4% and 25.5% TAR in soils LUFA 2.2, Li 10, LUFA 2.3 and LUFA 5 M, respectively.

Table 7.1.1.1-10: Radio-HPLC analysis of the soil extracts after treatment of soil LUFA 2.2 with ^{14}C-Iprodione and aerobic incubation at 20°C [% TAR]

DAT	207099 t_R ~ 22.9	85831 t_R ~ 28.4	BAS610F t_R ~33.7	5079626 t_R ~ 35.8	5079618 t_R ~ 37.6	5079647 t_R ~ 39.3	Sum others*	Total
Acetonitrile extracts								
0 (rep 1)	-	-	96.8	-	-	-	1.4	98.3
0 (rep 2)	-	-	94.5	-	-	-	2.0	96.4
0 (mean)	**-**	**-**	**95.6**	**-**	**-**	**-**	**1.7**	**97.3**
3 (rep 1)	1.2	0.4	90.5	1.2	-	-	1.3	94.7
3 (rep 2)	1.5	-	87.8	1.5	0.5	0.5	0.9	92.7
3 (mean)	**1.4**	**0.2**	**89.1**	**1.4**	**0.2**	**0.2**	**1.1**	**93.7**
7 (rep 1)	1.9	1.5	85.2	1.1	1.0	0.5	1.1	92.3
7 (rep 2)	1.7	1.7	84.6	2.8	1.0	0.9	0.8	93.5
7 (mean)	**1.8**	**1.6**	**84.9**	**2.0**	**1.0**	**0.7**	**0.9**	**92.9**
14 (rep 1)	2.2	1.6	79.4	1.1	1.2	1.3	1.0	87.8
14 (rep 2)	1.5	1.8	74.8	1.8	1.2	1.6	1.5	84.2
14 (mean)	**1.9**	**1.7**	**77.1**	**1.4**	**1.2**	**1.4**	**1.2**	**86.0**
27 (rep 1)	1.9	1.2	72.0	0.9	1.7	1.4	0.7	79.8
27 (rep 2)	2.7	1.4	71.1	0.6	1.5	1.4	0.7	79.4
27 (mean)	**2.3**	**1.3**	**71.5**	**0.8**	**1.6**	**1.4**	**0.7**	**79.6**
41 (rep 1)	2.3	2.8	64.8	0.6	1.9	2.0	0.2	74.7
41 (rep 2)	1.8	2.8	64.3	1.0	1.7	2.0	1.0	74.5
41 (mean)	**2.1**	**2.8**	**64.6**	**0.8**	**1.8**	**2.0**	**0.6**	**74.6**
58 (rep 1)	1.4	2.1	54.4	0.4	1.6	1.9	1.3	63.0
58 (rep 2)	1.6	1.8	52.1	0.7	1.4	1.8	0.6	60.1
58 (mean)	**1.5**	**2.0**	**53.2**	**0.5**	**1.5**	**1.9**	**0.9**	**61.5**
92 (rep 1)	2.3	2.4	44.7	0.3	2.4	2.1	0.6	54.8
92 (rep 2)	2.0	3.0	43.8	0.4	2.2	2.2	1.2	54.9
92 (mean)	**2.2**	**2.7**	**44.3**	**0.4**	**2.3**	**2.2**	**0.9**	**54.9**
120 (rep 1)	2.0	1.6	42.1	-	3.0	2.1	0.4	51.2
120 (rep 2)	2.4	2.5	38.9	-	2.8	1.6	2.0	50.3
120 (mean)	**2.2**	**2.0**	**40.5**	**-**	**2.9**	**1.9**	**1.2**	**50.7**
Water+acetonitrile extracts								
0 (rep 1)	-	-	1.5	0.1	0.0	0.0	0.1	1.7
0 (rep 2)	-	-	1.8	0.1	0.0	0.0	0.1	2.1
0 (mean)	**-**	**-**	**1.6**	**0.1**	**0.0**	**0.0**	**0.1**	**1.9**
3 (rep 1)	0.1	0.0	2.4	1.2	0.1	0.1	0.2	4.0
3 (rep 2)	0.1	0.0	2.2	1.1	0.1	0.2	0.2	3.8
3 (mean)	**0.1**	**0.0**	**2.3**	**1.1**	**0.1**	**0.1**	**0.2**	**3.9**
7 (rep 1)	0.1	0.1	2.4	1.2	0.1	0.3	0.2	4.4
7 (rep 2)	0.1	0.1	2.5	1.1	0.1	0.4	0.1	4.4
7 (mean)	**0.1**	**0.1**	**2.5**	**1.1**	**0.1**	**0.4**	**0.2**	**4.4**
14 (rep 1)	0.2	0.1	2.7	0.8	0.2	0.5	0.2	4.7
14 (rep 2)	0.2	0.1	2.3	0.7	0.2	0.6	0.3	4.3
14 (mean)	**0.2**	**0.1**	**2.5**	**0.8**	**0.2**	**0.5**	**0.2**	**4.5**
27 (rep 1)	0.2	0.1	2.5	0.7	0.3	0.2	0.2	4.3
27 (rep 2)	0.3	0.1	2.4	0.7	0.3	0.2	0.2	4.3
27 (mean)	**0.2**	**0.1**	**2.4**	**0.7**	**0.3**	**0.2**	**0.2**	**4.3**
41 (rep 1)	0.2	0.3	2.6	0.7	0.4	0.2	0.5	5.0
41 (rep 2)	0.3	0.3	2.3	0.8	0.4	0.2	0.4	4.7
41 (mean)	**0.3**	**0.3**	**2.5**	**0.8**	**0.4**	**0.2**	**0.4**	**4.9**
58 (rep 1)	0.2	0.2	2.5	0.6	0.6	0.2	0.3	4.6
58 (rep 2)	0.3	0.1	2.7	0.5	0.5	0.2	0.3	4.7
58 (mean)	**0.2**	**0.1**	**2.6**	**0.6**	**0.6**	**0.2**	**0.3**	**4.6**
92 (rep 1)	0.5	0.7	4.2	0.5	0.7	0.3	0.4	7.3
92 (rep 2)	0.4	0.6	4.2	0.4	0.7	0.4	0.4	7.1
92 (mean)	**0.5**	**0.7**	**4.2**	**0.4**	**0.7**	**0.4**	**0.4**	**7.2**
120 (rep 1)	0.4	0.9	3.9	0.3	0.8	0.4	0.6	7.3
120 (rep 2)	0.4	0.9	3.3	0.3	0.8	0.4	0.9	7.2
120 (mean)	**0.4**	**0.9**	**3.6**	**0.3**	**0.8**	**0.4**	**0.7**	**7.2**

Table 7.1.1.1-10 continued

DAT	207099 t_R ~ 22.9	85831 t_R ~ 28.4	BAS610F t_R ~33.7	5079626 t_R ~ 35.8	5079618 t_R ~ 37.6	5079647 t_R ~ 39.3	Sum others*	Total
Total extracts								
0 (rep 1)	-	-	98.3	0.1	0.0	0.0	1.5	100.0
0 (rep 2)	-	-	96.2	0.1	0.0	0.0	2.1	98.5
0 (mean)	**-**	**-**	**97.3**	**0.1**	**0.0**	**0.0**	**1.8**	**99.2**
3 (rep 1)	1.3	0.4	92.9	2.4	0.1	0.1	1.5	98.7
3 (rep 2)	1.6	0.0	90.0	2.6	0.5	0.6	1.1	96.5
3 (mean)	**1.5**	**0.2**	**91.4**	**2.5**	**0.3**	**0.4**	**1.3**	**97.6**
7 (rep 1)	2.0	1.5	87.6	2.3	1.1	0.8	1.3	96.7
7 (rep 2)	1.8	1.8	87.1	3.9	1.1	1.3	0.9	98.0
7 (mean)	**1.9**	**1.7**	**87.3**	**3.1**	**1.1**	**1.0**	**1.1**	**97.3**
14 (rep 1)	2.4	1.6	82.1	1.9	1.4	1.7	1.2	92.4
14 (rep 2)	1.7	1.9	77.1	2.5	1.4	2.2	1.8	88.5
14 (mean)	**2.0**	**1.8**	**79.6**	**2.2**	**1.4**	**2.0**	**1.5**	**90.5**
27 (rep 1)	2.1	1.3	74.5	1.6	1.9	1.6	0.9	84.0
27 (rep 2)	2.9	1.6	73.5	1.4	1.8	1.6	0.9	83.6
27 (mean)	**2.5**	**1.5**	**74.0**	**1.5**	**1.9**	**1.6**	**0.9**	**83.8**
41 (rep 1)	2.6	3.1	67.4	1.3	2.3	2.2	0.6	79.6
41 (rep 2)	2.1	3.1	66.7	1.8	2.1	2.2	1.3	79.3
41 (mean)	**2.4**	**3.1**	**67.0**	**1.5**	**2.2**	**2.2**	**1.0**	**79.4**
58 (rep 1)	1.6	2.3	56.8	1.0	2.2	2.1	1.6	67.5
58 (rep 2)	1.8	1.9	54.8	1.2	2.0	2.1	0.9	64.7
58 (mean)	**1.7**	**2.1**	**55.8**	**1.1**	**2.1**	**2.1**	**1.2**	**66.1**
92 (rep 1)	2.8	3.2	48.9	0.8	3.1	2.5	1.0	62.2
92 (rep 2)	2.5	3.6	48.0	0.9	2.9	2.6	1.6	62.0
92 (mean)	**2.6**	**3.4**	**48.4**	**0.8**	**3.0**	**2.5**	**1.3**	**62.1**
120 (rep 1)	2.5	2.5	46.0	0.3	3.8	2.5	1.0	58.5
120 (rep 2)	2.8	3.4	42.3	0.3	3.6	2.1	2.9	57.4
120 (mean)	**2.6**	**3.0**	**44.1**	**0.3**	**3.7**	**2.3**	**1.9**	**58.0**

TAR = total applied radioactivity (100% = 5.192 mg/kg)
DAT = days after treatment
t_R = retention time [min]
rep = replicate
mean = mean value of replicates
* = each single peak ≤ 0.9% TAR
- = no peak detected

Table 7.1.1.1-11: Radio-HPLC analysis of the soil extracts after treatment of soil Li 10 with ^{14}C-iprodione and aerobic incubation at 20°C [% TAR]

DAT	207099 t_R ~ 22.9	85831 t_R ~ 28.5	BAS610F t_R ~33.6	5079626 t_R ~ 35.7	5079618 t_R ~ 37.4	5079647 t_R ~ 39.3	Sum others*	Total
Acetonitrile extracts								
0 (rep 1)	-	-	92.9	0.1	-	-	3.3	96.3
0 (rep 2)	-	-	92.8	1.2	-	-	3.8	97.8
0 (mean)	**-**	**-**	**92.9**	**0.7**	**-**	**-**	**3.5**	**97.1**
3 (rep 1)	**1.3**	**0.5**	**83.2**	**2.6**	**0.9**	**0.8**	**2.6**	**91.8**
7 (rep 1)	**1.9**	**1.2**	**78.6**	**1.8**	**0.8**	**2.3**	**0.9**	**87.6**
14 (rep 1)	**1.8**	**1.4**	**75.0**	**2.6**	**1.1**	**4.0**	**0.9**	**86.9**
24 (rep 1)	**5.6**	**4.7**	**60.0**	**1.6**	**1.5**	**5.3**	**0.5**	**79.3**
42 (rep 2)	**2.0**	**4.7**	**49.3**	**2.1**	**2.1**	**7.4**	**0.4**	**68.0**
59 (rep 1)	1.8	7.2	33.5	0.8	2.6	7.4	0.3	53.6
59 (rep 2)	1.2	3.4	44.6	0.2	2.6	8.2	0.5	60.7
59 (mean)	**1.5**	**5.3**	**39.1**	**0.5**	**2.6**	**7.8**	**0.4**	**57.2**
91 (rep 1)	**0.9**	**4.5**	**23.4**	**0.3**	**2.9**	**7.0**	**0.4**	**39.4**
120 (rep 1)	0.8	5.4	4.6	0.3	3.5	5.6	0.6	20.8
120 (rep 2)	1.3	4.8	6.1	0.2	4.0	7.2	1.0	24.7
120 (mean)	**1.0**	**5.1**	**5.4**	**0.3**	**3.8**	**6.4**	**0.8**	**22.8**
Water+acetonitrile extracts								
0 (rep 1)	-	-	1.6	0.2	0.0	0.0	0.2	2.0
0 (rep 2)	-	-	2.0	0.3	0.0	0.0	0.1	2.5
0 (mean)	**-**	**-**	**1.8**	**0.3**	**0.0**	**0.0**	**0.1**	**2.3**
3 (rep 1)	**0.1**	**-**	**2.4**	**3.4**	**0.1**	**0.2**	**0.2**	**6.4**
7 (rep 1)	**0.1**	**0.0**	**2.5**	**3.5**	**0.2**	**0.7**	**0.1**	**7.1**
14 (rep 1)	**0.2**	**0.2**	**2.6**	**3.0**	**0.2**	**0.6**	**0.2**	**7.0**
24 (rep 1)	**0.4**	**0.4**	**2.2**	**2.6**	**0.3**	**0.3**	**0.4**	**6.6**
42 (rep 2)	**0.2**	**0.4**	**1.7**	**1.9**	**0.4**	**0.8**	**0.4**	**5.9**
59 (rep 1)	0.5	0.6	2.2	1.5	0.7	0.8	0.8	7.2
59 (rep 2)	0.3	0.4	3.0	2.6	0.9	1.2	0.3	8.7
59 (mean)	**0.4**	**0.5**	**2.6**	**2.1**	**0.8**	**1.0**	**0.6**	**7.9**
91 (rep 1)	**0.7**	**4.9**	**3.4**	**0.9**	**1.1**	**1.7**	**0.2**	**12.9**
120 (rep 1)	0.9	7.4	2.0	0.4	1.4	1.3	1.2	14.6
120 (rep 2)	0.8	7.5	2.2	0.6	1.5	1.4	0.6	14.6
120 (mean)	**0.9**	**7.5**	**2.1**	**0.5**	**1.5**	**1.3**	**0.9**	**14.6**
Total extracts								
0 (rep 1)	-	-	94.5	0.3	0.0	0.0	3.4	98.3
0 (rep 2)	-	-	94.8	1.5	0.0	0.0	3.9	100.3
0 (mean)	**-**	**-**	**94.7**	**0.9**	**0.0**	**0.0**	**3.7**	**99.3**
3 (rep 1)	**1.3**	**0.5**	**85.6**	**6.0**	**1.0**	**1.0**	**2.8**	**98.2**
7 (rep 1)	**2.0**	**1.3**	**81.1**	**5.4**	**1.0**	**3.0**	**1.0**	**94.7**
14 (rep 1)	**2.1**	**1.7**	**77.6**	**5.6**	**1.3**	**4.6**	**1.1**	**93.9**
24 (rep 1)	**6.0**	**5.1**	**62.2**	**4.3**	**1.8**	**5.7**	**0.8**	**86.0**
42 (rep 2)	**2.3**	**5.1**	**51.0**	**4.0**	**2.5**	**8.2**	**0.9**	**73.9**
59 (rep 1)	2.3	7.8	35.7	2.4	3.3	8.2	1.1	60.8
59 (rep 2)	1.5	3.8	47.7	2.7	3.5	9.4	0.8	69.4
59 (mean)	**1.9**	**5.8**	**41.7**	**2.6**	**3.4**	**8.8**	**0.9**	**65.1**
91 (rep 1)	**1.5**	**9.4**	**26.8**	**1.3**	**3.9**	**8.7**	**0.6**	**52.3**
120 (rep 1)	1.7	12.9	6.6	0.7	5.0	6.8	1.7	35.4
120 (rep 2)	2.1	12.3	8.3	0.8	5.5	8.7	1.6	39.4
120 (mean)	**1.9**	**12.6**	**7.5**	**0.7**	**5.2**	**7.7**	**1.6**	**37.4**

TAR = total applied radioactivity (100% = 5.176 mg/kg)
DAT = days after treatment
t_R = retention time [min]
rep = replicate
mean = mean value of replicates
* = each single peak < 1.4% TAR
- = no peak detected

Table 7.1.1.1-12: Radio-HPLC analysis of the soil extracts after treatment of soil LUFA 2.3 with ^{14}C-iprodione and aerobic incubation at 20°C [% TAR]

DAT	207099 t_R ~ 22.8	85831 t_R ~ 28.4	BAS610F t_R ~33.6	5079626 t_R ~ 35.7	5079618 t_R ~ 37.3	5079647 t_R ~ 39.2	Sum others*	Total
Acetonitrile extracts								
0 (rep 1)	-	-	89.3	0.1	-	-	2.9	92.4
0 (rep 2)	0.4	-	91.0	1.5	-	-	1.8	94.7
0 (mean)	**0.2**	**-**	**90.2**	**0.8**	**-**	**-**	**2.4**	**93.5**
3 (rep 1)	**1.5**	**0.6**	**68.3**	**2.5**	**1.0**	**1.2**	**1.1**	**76.3**
7 (rep 1)	**2.0**	**2.5**	**53.6**	**3.4**	**1.8**	**4.3**	**0.4**	**68.0**
14 (rep 1)	**4.1**	**4.3**	**39.0**	**2.5**	**2.3**	**7.6**	**0.8**	**60.5**
23 (rep 1)	**2.2**	**7.9**	**25.2**	**2.0**	**2.6**	**8.7**	**0.6**	**49.3**
43 (rep 1)	**0.9**	**6.0**	**15.5**	**1.3**	**3.0**	**8.5**	**0.4**	**35.6**
57 (rep 1)	0.5	6.2	12.2	0.8	3.2	7.8	0.8	31.5
57 (rep 2)	0.5	5.5	12.5	0.7	2.8	7.5	0.5	30.0
57 (mean)	**0.5**	**5.9**	**12.4**	**0.8**	**3.0**	**7.6**	**0.7**	**30.7**
91 (rep 1)	**0.6**	**4.0**	**8.6**	**0.9**	**4.1**	**6.8**	**0.6**	**25.7**
120 (rep 1)	0.5	2.6	5.6	0.6	4.8	5.7	0.6	20.5
120 (rep 2)	0.5	4.5	4.2	0.4	3.8	4.5	1.4	19.3
120 (mean)	**0.5**	**3.6**	**4.9**	**0.5**	**4.3**	**5.1**	**1.0**	**19.9**
Water+acetonitrile extracts								
0 (rep 1)	-	-	3.3	1.1	0.0	0.2	0.2	4.9
0 (rep 2)	-	-	3.4	1.1	0.0	0.3	0.1	5.0
0 (mean)	**-**	**-**	**3.3**	**1.1**	**0.0**	**0.3**	**0.1**	**4.9**
3 (rep 1)	**0.2**	**0.0**	**4.6**	**9.7**	**0.2**	**1.0**	**0.6**	**16.5**
7 (rep 1)	**0.5**	**0.3**	**4.7**	**10.0**	**0.4**	**2.5**	**0.6**	**19.1**
14 (rep 1)	**0.8**	**0.2**	**4.0**	**8.4**	**0.6**	**2.1**	**0.3**	**16.5**
23 (rep 1)	**0.6**	**1.4**	**2.8**	**6.0**	**0.7**	**1.4**	**0.5**	**13.5**
43 (rep 1)	**0.5**	**1.9**	**3.1**	**3.7**	**1.2**	**2.3**	**0.7**	**13.4**
57 (rep 1)	0.7	4.3	3.8	3.8	1.9	3.0	0.9	18.3
57 (rep 2)	0.7	4.1	4.3	3.8	1.8	3.1	0.9	18.7
57 (mean)	**0.7**	**4.2**	**4.1**	**3.8**	**1.8**	**3.0**	**0.9**	**18.5**
91 (rep 1)	**0.7**	**4.9**	**3.3**	**1.7**	**2.1**	**3.8**	**-**	**16.5**
120 (rep 1)	0.6	2.8	2.4	1.5	2.6	2.9	2.2	15.1
120 (rep 2)	0.9	3.0	2.4	1.2	2.7	2.8	2.4	15.3
120 (mean)	**0.7**	**2.9**	**2.4**	**1.4**	**2.7**	**2.9**	**2.3**	**15.2**
Total extracts								
0 (rep 1)	-	-	92.7	1.3	0.0	0.2	3.1	97.3
0 (rep 2)	0.4	-	94.4	2.7	0.0	0.3	1.9	99.6
0 (mean)	**0.2**	**-**	**93.5**	**2.0**	**0.0**	**0.3**	**2.5**	**98.5**
3 (rep 1)	**1.7**	**0.7**	**73.0**	**12.2**	**1.2**	**2.2**	**1.7**	**92.8**
7 (rep 1)	**2.5**	**2.8**	**58.4**	**13.4**	**2.2**	**6.8**	**1.0**	**87.1**
14 (rep 1)	**4.9**	**4.5**	**43.0**	**11.0**	**2.9**	**9.7**	**1.1**	**77.1**
23 (rep 1)	**2.8**	**9.3**	**28.0**	**8.0**	**3.4**	**10.1**	**1.1**	**62.8**
43 (rep 1)	**1.4**	**7.9**	**18.6**	**5.0**	**4.2**	**10.8**	**1.1**	**49.0**
57 (rep 1)	1.1	10.5	16.1	4.6	5.1	10.8	1.7	49.8
57 (rep 2)	1.3	9.6	16.8	4.6	4.6	10.5	1.4	48.7
57 (mean)	**1.2**	**10.1**	**16.4**	**4.6**	**4.8**	**10.6**	**1.5**	**49.3**
91 (rep 1)	**1.3**	**8.9**	**11.9**	**2.6**	**6.2**	**10.6**	**0.6**	**42.2**
120 (rep 1)	1.1	5.5	7.9	2.2	7.4	8.7	2.8	35.6
120 (rep 2)	1.4	7.5	6.6	1.6	6.5	7.2	3.7	34.6
120 (mean)	**1.3**	**6.5**	**7.3**	**1.9**	**6.9**	**8.0**	**3.3**	**35.1**

TAR = total applied radioactivity (100% = 5.244 mg/kg)
DAT = days after treatment
t_R = retention time [min]
rep = replicate
mean = mean value of replicates
* = each single peak < 1.4% TAR
- = no peak detected

Table 7.1.1.1-13: Radio-HPLC analysis of the soil extracts after treatment of soil LUFA 5M with ^{14}C-iprodione and aerobic incubation at 20°C [% TAR]

DAT	207099 t_R ~ 23.0	85831 t_R ~ 28.6	BAS610F t_R ~33.6	5079626 t_R ~ 35.7	5079618 t_R ~ 37.3	5079647 t_R ~ 39.1	Sum others*	Total
Acetonitrile extracts								
0 (rep 1)	-	-	89.0	2.7	0.4	-	1.9	94.0
0 (rep 2)	-	-	91.2	3.1	-	-	0.6	94.8
0 (mean)	**-**	**-**	**90.1**	**2.9**	**0.2**	**-**	**1.2**	**94.4**
3 (rep 1)	**-**	**-**	**62.5**	**3.5**	**0.9**	**3.0**	**-**	**69.9**
7 (rep 1)	**0.4**	**0.9**	**43.6**	**2.2**	**1.0**	**7.9**	**1.3**	**57.3**
14 (rep 1)	**0.6**	**2.2**	**32.1**	**2.9**	**1.4**	**14.7**	**0.2**	**54.3**
23 (rep 1)	**0.5**	**4.3**	**23.3**	**0.9**	**1.7**	**18.2**	**0.8**	**49.8**
43 (rep 1)	**-**	**5.3**	**15.3**	**0.7**	**2.1**	**18.5**	**0.8**	**42.7**
57 (rep 1)	0.0	6.8	11.8	0.3	3.0	16.0	0.4	38.4
57 (rep 2)	0.2	6.0	11.7	1.0	3.6	15.9	0.1	38.5
57 (mean)	**0.1**	**6.4**	**11.7**	**0.6**	**3.3**	**15.9**	**0.3**	**38.4**
91 (rep 1)	**0.1**	**2.9**	**6.3**	**0.7**	**4.5**	**8.9**	**1.2**	**24.6**
120 (rep 1)	0.3	1.9	3.2	0.4	4.3	4.2	1.9	16.1
120 (rep 2)	0.2	1.8	3.6	0.2	4.6	4.8	1.0	16.1
120 (mean)	**0.2**	**1.8**	**3.4**	**0.3**	**4.5**	**4.5**	**1.4**	**16.1**
Water+acetonitrile extracts								
0 (rep 1)	-	0.0	2.8	1.6	0.0	0.5	0.1	5.2
0 (rep 2)	-	-	2.1	1.3	0.1	0.5	0.2	4.1
0 (mean)	**-**	**0.0**	**2.5**	**1.4**	**0.1**	**0.5**	**0.2**	**4.6**
3 (rep 1)	**-**	**-**	**3.9**	**20.8**	**-**	**3.6**	**0.5**	**28.8**
7 (rep 1)	**-**	**0.1**	**4.8**	**23.3**	**0.2**	**6.8**	**0.4**	**35.5**
14 (rep 1)	**-**	**0.2**	**4.2**	**18.5**	**0.3**	**7.2**	**0.2**	**30.6**
23 (rep 1)	**0.2**	**0.4**	**3.3**	**19.2**	**0.6**	**4.5**	**0.4**	**28.5**
43 (rep 1)	**0.3**	**0.9**	**2.9**	**11.8**	**1.0**	**5.4**	**0.2**	**22.5**
57 (rep 1)	0.3	0.8	2.2	9.6	0.9	3.7	0.4	17.9
57 (rep 2)	0.3	0.8	2.0	8.1	0.8	3.3	0.3	15.7
57 (mean)	**0.3**	**0.8**	**2.1**	**8.9**	**0.9**	**3.5**	**0.4**	**16.8**
91 (rep 1)	**0.5**	**3.3**	**1.4**	**2.7**	**1.1**	**3.8**	**2.3**	**15.1**
120 (rep 1)	0.7	2.8	1.0	1.5	1.7	2.5	3.0	13.1
120 (rep 2)	0.7	1.9	1.1	2.1	1.7	3.4	2.6	13.6
120 (mean)	**0.7**	**2.4**	**1.1**	**1.8**	**1.7**	**2.9**	**2.8**	**13.3**
Total extracts								
0 (rep 1)	-	0.0	91.8	4.3	0.5	0.5	2.0	99.2
0 (rep 2)	-	-	93.2	4.3	0.1	0.5	0.8	98.9
0 (mean)	**-**	**0.0**	**92.5**	**4.3**	**0.3**	**0.5**	**1.4**	**99.0**
3 (rep 1)	**-**	**-**	**66.4**	**24.3**	**0.9**	**6.6**	**0.5**	**98.7**
7 (rep 1)	**0.4**	**1.0**	**48.4**	**25.5**	**1.2**	**14.7**	**1.6**	**92.8**
14 (rep 1)	**0.6**	**2.5**	**36.3**	**21.4**	**1.7**	**21.9**	**0.3**	**84.9**
23 (rep 1)	**0.7**	**4.7**	**26.5**	**20.1**	**2.3**	**22.7**	**1.2**	**78.3**
43 (rep 1)	**0.3**	**6.2**	**18.2**	**12.5**	**3.2**	**23.9**	**1.0**	**65.2**
57 (rep 1)	0.4	7.7	14.0	10.0	3.9	19.6	0.8	56.4
57 (rep 2)	0.5	6.8	13.6	9.1	4.4	19.2	0.5	54.1
57 (mean)	**0.5**	**7.2**	**13.8**	**9.5**	**4.1**	**19.4**	**0.6**	**55.2**
91 (rep 1)	**0.7**	**6.2**	**7.7**	**3.4**	**5.6**	**12.7**	**3.6**	**39.7**
120 (rep 1)	0.9	4.7	4.2	1.9	6.0	6.7	4.9	29.2
120 (rep 2)	0.8	3.7	4.7	2.3	6.3	8.2	3.6	29.7
120 (mean)	**0.9**	**4.2**	**4.5**	**2.1**	**6.2**	**7.4**	**4.2**	**29.4**

TAR = total applied radioactivity (100% = 5.243 mg/kg)
DAT = days after treatment
t_R = retention time [min]
rep = replicate
mean = mean value of replicates
* = each single peak < 0.8% TAR
- = no peak detected

Under aerobic conditions, the metabolic pathway for the soil degradation of iprodione involved primarily the opening of the hydantoin ring to form the carboxylic acid Reg.No. 5079626 (RP 35606), which can re-cyclise to the rearrangement product Reg.No. 5079647 (RP 30228) or form the carbamoyl urea Reg.No. 5079618 (RP 36221). Subsequent degradation leads to 3,5-dichloroaniline (Reg.No. 85831, RP 32596) which is ultimately mineralised to CO_2 or incorporated into bound residues. A minor degradation path for iprodione includes the formation of the dioxoimidazolidine Reg.No. 207099 (RP 25040), which should also be subject of further degradation.

Kinetic analysis and calculation of DT_{50} and DT_{90} values for iprodione was performed following the recommendations of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. The DFOP model provided the best visual and statistical fit to the observed data from the soils LUFA 2.2 and 5M. For the observed data from soils Li 10 and LUFA 2.3, the SFO and FOMC models provided the best visual and statistical fit, respectively. The estimated DT_{50} values of iprodione were calculated to be 91.1, 43.5, 9.6 and 6.3 days in soil LUFA 2.2, Li10, LUFA 2.3 and LUFA 5M, respectively. The DT_{90} values were 376.9, 144.4, 99.2 and 73.7 days in soil LUFA 2.2, Li10, LUFA 2.3 and LUFA 5M, respectively.

Table 7.1.1.1-14: Summary of kinetic evaluation

Soil	pH ($CaCl_2$)	DT_{50} [d]	Best-fit kinetic	Chi^2
LUFA 2.2	5.5	91.1	DFOP	2.3
Li 10	6.2	43.5	SFO	5.9
LUFA 2.3	6.7	9.6	FOMC	3.2
LUFA 5M	7.3	6.3	DFOP	2.2

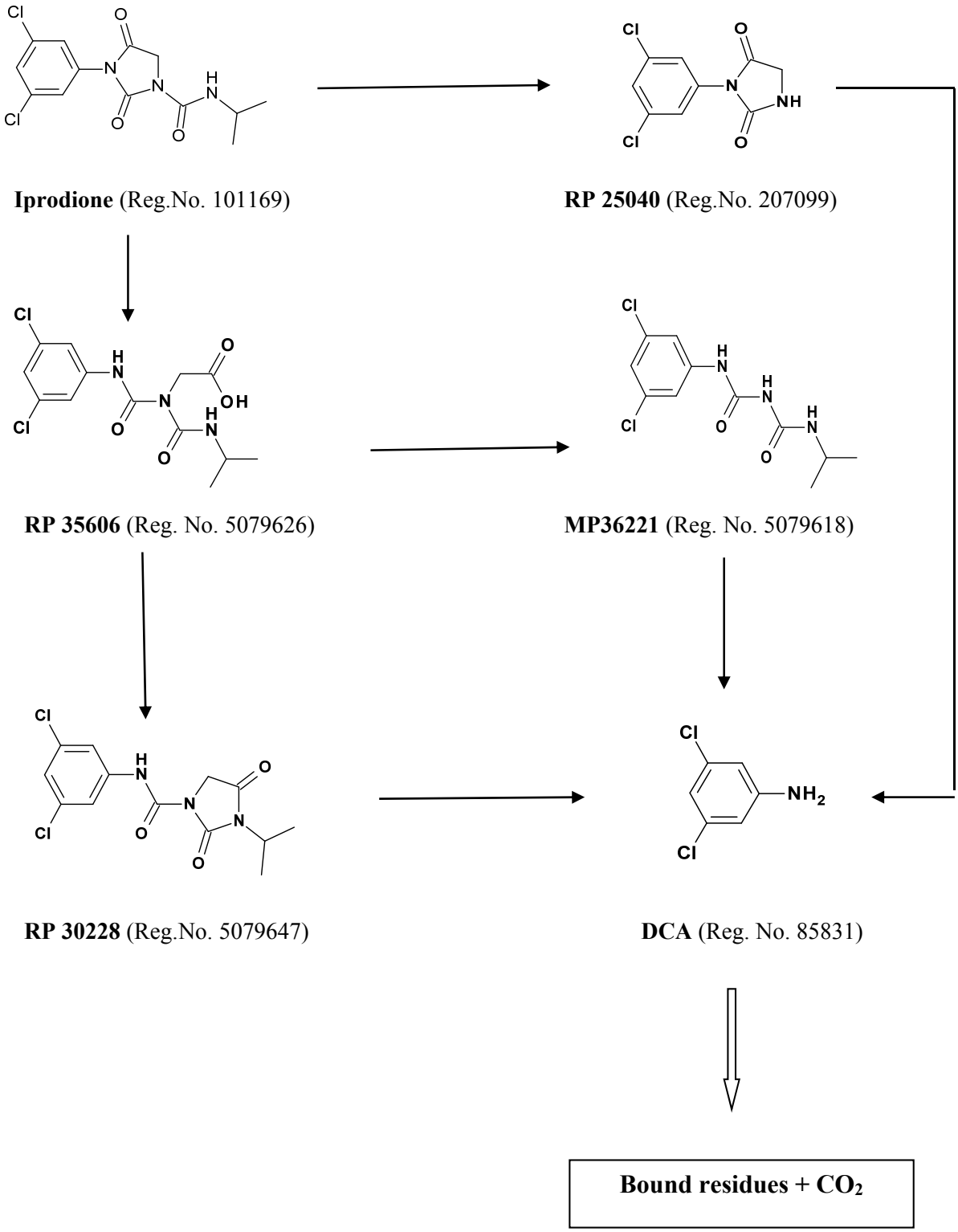
III. CONCLUSION

Iprodione was rapidly degraded in the tested soils. Degradation of iprodione proceeds primarily via opening of the imidazolidindione ring followed by further degradation steps ultimately leading to the formation of CO_2 and incorporation of the transformation products into bound residues. Several metabolites were detected in the soil extracts and identified as M610F004 (Reg. No. 207099, RP 25040), M610F012 (Reg. No. 85831, RP 32596, DCA), M610F014 (Reg. No. 5079626, RP 35606), M610F002 (Reg. No. 5079618, RP 36221) and M610F001 (Reg. No. 5079647, RP 30228). Expect in the soil LUFA 2.2, these metabolites reached concentrations above 5% TAR.

Characterisation of the non-extractable radioactive residues (NER) revealed that the major portion of radioactivity was tightly bound to the soil matrix or part of the humic acid fraction. Since iprodione was detectable only in very low amounts in the NER, it is assumed that no significant amounts of iprodione can be released from the bound residues even with harsh and artificial extraction methods. The proposed route of degradation of iprodione in aerobic soil is presented in Figure 7.1.1.1-1.

A detailed kinetic analysis of this study including all metabolites is given in Budde [*Budde (2014b) – 2013/1311391, CA 7.1.2.1.1/2*] in chapter 7.1.2, CA 7.1.2.1.1./2.

Figure 7.1.1.1-1: Proposed route of degradation of iprodione in aerobic soil



Supplementary information (Study presented in the original Annex II Dossier of 1995):

Report: Waring, A.R. (1993): (^{14}C)-iprodione: aerobic soil metabolism. Report Hazleton Europe 68/132-1015 of December 20, 1993. C022365
GLP: Yes

This study was carried out to meet the requirements of the US EPA Pesticide Assessment Guidelines. A sandy loam soil which had an organic matter content of 1.28% and a pH of 6.08 was treated with iprodione at a rate of 10 mg/kg, equivalent to 10 kg/ha. The incubation was carried out at about 25°C over a period of 276 days which was sufficient to define the pattern of decline of parent compound and the patterns of formation and decline of degradates. Traps for volatile compounds and carbon dioxide were included in the test system.

Results: The results show that iprodione has a fairly short half-time under aerobic soil conditions (half-life of about 10 days calculated on data up to about 2 months). Over the course of the study the amount of parent compound detected fell from >98% of applied material to <1%. The main degradation products were RP 30228, RP 32596 and RP 25040 which respectively accounted for up to 6%, 9% and 8% of applied radioactivity at various time points. An additional nine minor metabolites corresponding chromatographically to known standards (RP 36221, RP 35606, RP 36116, RP 37176, RP 36112, RP 32490, RP 36118, RP 36119 and RP 36114), individually accounted for < 4% of applied radioactivity. Small quantities of radiolabelled carbon dioxide were detected (approximately 5% of applied radioactivity by day 276) and these were still increasing when the study was terminated.

The quantities of non-extractable (bound) residues increased throughout the study, rising particularly sharply between the 14 and 30 day time points, to account for about 75% of applied material at the conclusion of the study. About 20% of these bound residues were associated with the fulvic acid fraction of the soil, 30% with the humic acid and 50% with the humin.

Conclusion: Iprodione has a short half-life under the conditions used. Bound residues were an important factor in the disappearance of parent compound and carbon dioxide was the ultimate degradation product.

The kinetic analysis of this study was repeated by Szegedi [*Szegedi (2014) – 2013/1311394, CA 7.1.2.1.1/3*] considering latest guidance documents. The determined half-life (trigger $DegT_{50}$) is 16.5 d. Details are given in chapter 7.1.2, CA 7.1.2.1.1./2.

Supplementary information – Scientific literature

It is known from literature that the degradation rate of iprodione in soil increases, when soils are previously treated with iprodione. (See Walker in Pestic. Sci. 1987, **21**, 233-240 and Pestic. Sci. 1987 *21*, 219-231) Mercadier (1996) investigated the effect of an arthobacter strain on the degradation of iprodione and Vanni (2000) the metabolism in compost.

Report: CA 7.1.1.1/2
Mercadier C. et al., 1996a
Metabolism of Iprodione in adapted and non-adapted soils; effect of soil inoculation with an Iprodione-degrading Arthrobacter strain
1996/1006260
Guidelines: none
GLP: no

Executive Summary

The aim of the study was to investigate iprodione degradation pathways in adapted and non-adapted soils. Moreover, the effect of the addition of *Arthrobacter* sp. to non-adapted soils was studied. Iprodione degraded more rapidly in soils previously treated with iprodione than in previously untreated samples of the same soils. Addition of an iprodione-degrading *Arthrobacter* sp. culture to a previously untreated soil enhanced the degradation rate of the fungicide. In previously untreated soils, chemical and biological transformations of iprodione led to different products. In treated soils as well as in untreated soils enriched with *Arthrobacter* sp., the product obtained from the hydrolysis of exocyclic urea function of iprodione was transformed initially into 3,5-dichlorophenylurea acetic acid, which was then degraded to 3,5-dichloroaniline.

I. MATERIALS AND METHODS

1. Test material

The following materials were used:
- Iprodione in the form of a commercial 500 g a.s. kg^{-1} wettable powder formulation (Rovral)
- Commercially available chemicals:
    - iprodione isomer isopropyl-3-N-(3,5-dichlorophenyl)2,4-dioxoimidazolidine-1-carboxamide (metabolite VI)
    - 3,5-dichloroaniline (metabolite IV)
- Obtained by chemical synthesis:
    - 3-isopropyl-carbamoyl(3,5-dichlorophenyl)-5-hydantoic acid (metabolite V)
    - N-(3,5-dichlorophenyl)-2,4-dioxoimidazolidine (metabolite II)
    - 3,5-dichlorophenylurea acetic acid (metabolite III)

2. Soil

Soil samples were collected at two sites in southern France (Mandon and Pezilla) from a depth of 0-15 cm. Samples were taken from treated and untreated parts of each site. The treated soil from Mandon had received 8 applications of 0.75 kg a.s. ha^{-1} in the previous year, while the treated soil from Pezilla had been treated with 2.25 kg a.s. ha^{-1} a^{-1} for the previous 4 years.

3. Experimental conditions and analysis

An aqueous suspension of Rovral was added to treated as well as untreated soils to obtain a final concentration of 50 mg iprodione kg^{-1}. Samples were incubated at 20% moisture content (w/w of dry weight of soil) and 28°C. The same procedure was applied for the degradation studies with the metabolites N-(3,5-dichlorophenyl)-2,4-dioxoimidazolidine and 3,5-dichlorophenylurea acetic acid. Soils were extracted with acetonitrile:acetic acid (99:1) and analysed by HPLC.

4. *Arthrobacter* addition

Samples of non-adapted soil were inoculated with an iprodione-degrading *Arthrobacter* culture in a mineral medium supplemented with iprodione. The bacterial concentration was about 1.5 x 10^7 cells g^{-1} of soil. The soils were conditioned for 12 h at 28°C. Afterwards Rovral suspension was added to give a final concentration of 50 mg iprodione kg^{-1} in soil and the pesticide degradation was studied as described above.

II. RESULTS AND DISCUSSION

In adapted Mandon soil, iprodione rapidly decreased (85% disappeared after 8 h) and the metabolites N-(3,5-dichlorophenyl)-2,4-dioxoimidazolidine (II) and 3,5-dichlorophenylurea acetic acid (III) accumulated followed by formation of 3,5-dichloroaniline (IV). In non-adapted Mandon soil, metabolites II and III did not accumulate and the major transformation product was the isomer of iprodione. Addition of *Arthrobacter* to this untreated soil led to formation of increased amounts of metabolites II and III, with >75% of iprodione transformed after 72 h.

In adapted Pezilla soil, accumulation of metabolites II and III was very low and the formation of metabolite IV was rapid, with the metabolites appearing more readily when *Arthrobacter* was added to the non-treated soil. Only a small quantity of the isomer of iprodione was found in non-treated Pezilla soil.

The degradation rate of iprodione was greater in the treated than in the untreated soils for both Mandon and Pezilla soils (34 and 3 times greater, respectively). Inoculation with *Arthrobacter* increased the degradation rate in both untreated soils, with the DT_{50} in non-adapted soils being twice as high as the one found in non-adapted soils with *Arthrobacter* addition. In treated soils, the degradation of metabolites II and III coincided with the accumulation of a large amount of metabolite IV which then decreased without other metabolites being detected. DT_{50} values of metabolite III ranged from 6.5 to 15 hours.

Both biological and chemical degradation may be responsible for the transformation of metabolite II into metabolite III. By adding an *Arthrobacter* culture to untreated soils iprodione was quickly transformed. The degradation products detected in untreated soils inoculated with *Arthrobacter* were those obtained by the biological pathway as in the previously treated soils. It was therefore concluded that *Arthrobacter* sp. is involved in the first step of the degradation of iprodione to metabolite II. In both treated and untreated soils the formation of metabolite IV resulted from the degradation of metabolite III. Soil treatment with iprodione did not enhance the rate of this degradation step.

III. CONCLUSION

Iprodione degraded more rapidly in soils previously treated with iprodione than in previously untreated samples of the same soils. Addition of an iprodione-degrading *Arthrobacter* sp. culture to a previously untreated soil enhanced the degradation rate of the fungicide. In previously untreated soils, chemical and biological transformations of iprodione led to different products.

Report: CA 7.1.1.1/3
Vanni A. et al., 1999a
Determination and identification of metabolites of the fungicides Iprodione and Procymidone in compost
2000/1024061
Guidelines: none
GLP: no

Executive Summary
The aim of the study was to report the isolation and structural identification of the breakdown products of iprodione during the composting process. Verifications were made by comparison with scientific literature and by comparing the kinetic behaviour of every compound in the compost in a hydro-organic acid solution (55 % acetonitrile / 45 % sodium phosphate buffer pH 6.5). After taking into account the reaction kinetics in compost and hydro-organic solution, breakdown pathways are proposed for biodegradation.

MATERIALS AND METHODS

1. Test material

Iprodione (commercial formulation Rovral)

2. Study design

Biotic degradation
The added quantity of analyte was 20 µg per g dried compost (DC) considering the medium quantity of technical product to be applied on crops being 1.5 – 2 kg/ha and considering the characteristics of composting piles.
The pile was turned regularly and sampled at 0, 4, 11, 18, 25, 32, 43, 60, 94, 119, 151, 186 and 241 days after treatment. The compost samples were extracted using sonication disruption and acetonitrile extraction.

Abiotic degradation
Aliquots of 0.2 mL of a single standard solution at 500 µg/mL in acetonitrile were mixed with 0.01 M phosphate buffer at two different pH levels (i.e., pH 6 and pH 8.7). The final concentration of iprodione was 20 µg/mL. Aliquots where then incubated at two different temperatures (i.e., 35°C and 55°C) in a water bath in the dark and subsequently acidified to pH 3 to stop the reaction.

3. Analytics
The analysis of the extracts was executed by HPLC which was equipped with a UV-Vis/diode array detector and a mass detector. The absorption of the analytes was monitored at 210 nm. The limits of detection varied between the detection methods (LOD iprodione 0.035 – 0.05 mg/kg). The quantification of the peaks was carried out by an external standard method or based on the following assumptions: supposing that at a chosen wavelength the ε (molar absorptivity) of several formed metabolites remains practically equal to that of 3,5-DCA and analyte, one can assume that the Lambert-Beer equation could be applied to other breakdown products.

The kinetic behaviour of each compound was evaluated using the literature method [*Rodiguin & Rodiguina (1964). In: Schneider (Ed.), Consecutive Chemical Reactions. D. van Nostrand, New York, pp. 49 (Chapter V)*] which is based on parallel-consecutive reaction and well suited for this kind of kinetics.

RESULTS AND DISCUSSION

Biotic degradation
The rapid transformation of iprodione resulted in the complete transformation in its isomer 3-isopropyl-*N*-(3,5-dichlorophenyl)-2,4-dioxo-1-imidazolidine-carboxamide (RP 30228). After 70 days, 20 % of the isomer further degraded to other metabolites.

Abiotic degradation
The hydrolysis conditions which approached biotic conditions were pH 6 and 35°C. Iprodione rate constants were also influenced by pH and temperature variations, but more by pH rather than by temperature. Three breakdown products were identified, one being the isomer-like compound also formed during the composting process. The two new metabolites were identified as 3-isopropyl carbamoil-5-(3,5-dichlorophenyl)hydantoic acid (metabolite II, structure assignment seems incorrect: more likely RP 35606) and metabolite III. At the highest temperature (55°C) the presence of 3,5-DCA has additionally been determined.

Comparison of biotic and abiotic processes
For iprodione, hydrolysis is probably of minor importance for the composting process, while other transformations could prevail due to enzymatic activity of a particular microorganism. The analyte is completely converted to a more stable isomer which is formed with a rate constant in the same order to that of the biotic process. Simultaneously, metabolite II (reaction step 1) is formed under abiotic conditions, which is further transformed into metabolite III (reaction step 3). At pH 6 and 55°C metabolite II is transformed to 3,5-DCA (reaction step 4).
The first and third reaction steps are quite influenced by the increase of pH and temperature while the second step shows only a minor effect.

CONCLUSION

Iprodione will abiotically be degraded through parallel-consecutive reactions which comprise the two parallel chains, one composed of two simple reactions (steps 1 and 3) and the other composed of one simple reaction (reaction step 2). Biotic degradation of iprodione proceeds through a simple reaction which eventually leads to the complete conversion of iprodione into its own isomer.

CA 7.1.1.2 Anaerobic degradation

Not yet peer-reviewed study:

Report:	CA 7.1.1.2/1 Sopena-Vazquez F.,Bayer H., 2014a Anaerobic soil metabolism of BAS 610 F (Iprodione) 2013/1283134
Guidelines:	OECD 307, EPA 835.4200
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The anaerobic metabolism of [phenyl-U-^{14}C]-labelled iprodione (BAS 610 F) in a German loamy sand soil (USDA classification) was investigated under anaerobic conditions in darkness at 20ºC and 40% of maximum water holding capacity (MWHC), following 14 days of aerobic incubation. The nominal application rate was 5.3 mg/kg dry soil, which corresponds to a field application rate of 2.0 kg iprodione/ha (assuming a soil layer of 2.5 cm and bulk density of 1.5 g/cm^3).

Soil portions of 50.0 g dry soil equivalents (55.36 g of moist soil at 40% MWHC) were filled into 25 test vessels. A closed incubation system with continuous aeration (moistened air) was used together with an attached trapping system for collection of CO_2 and volatile organics. At 15 days after treatment (DAT), the aerobic soil samples were flooded with a water layer of about 2-3 cm depth and the incubation system was flushed with nitrogen instead of air, in order to establish and maintain anaerobic conditions. To monitor the conversion from aerobic to anaerobic conditions, the redox potential, the oxygen content and the pH of the test system were measured at each sampling time from 19 DAT onwards. Following treatment, duplicate soil samples were taken for analysis at 0, 14 (aerobic phase), 19, 26, 41, 55, 70, 90 and 119 DAT (anaerobic phase).

Concentrations of iprodione in soil decreased fast to an average of 12.8% TAR after 14 days of aerobic incubation. After switching from aerobic to anaerobic conditions, a slower degradation was observed with iprodione amounts decreasing to 3.0% TAR by the end of the study.

Two metabolites reached concentrations >5% of the total applied radioactivity, which were identified as Reg.No. 85831 (M610F012, RP 32596) and Reg.No. 5079647 (M610F001, RP 30228). Both metabolites were already formed during the aerobic phase of the study.

Characterisation of the non-extractable radioactive residues (NER) revealed that the predominant part of the radioactivity was tightly bound to the humins or incorporated into humic acids. Since iprodione was detectable only in trace amounts in the NER, it is assumed that no significant amounts of iprodione can be released from the bound residues even with harsh and artificial extraction methods.

Best-fit DT_{50} and DT_{90} values for iprodione, obtained with the Hockey-Stick model, were 32.24 and 107.10 days, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Internal code:	BAS 610 F
CAS Number.:	36734-19-7
Chemical name (IUPAC):	3-(3,5-dichlorophenyl)-N-isopropyl-2,4-dioxoimidazolidine-1-carboxamide
Molecular weight:	330.1697 g/mol (unlabelled)
Position of radiolabel:	phenyl-U-^{14}C
Specific activity:	6.69 MBq/mg (401400 dpm/µg)
Chemical purity:	94.9%
Radiochemical purity:	95.3%

2. Soil

The soil was classified as a loamy sand soil (USDA classification) from Germany, which has not been treated with any plant protection product within the previous 4 years. After collection, the soil was sieved (screen 2 mm), its moisture content adjusted to 40% of maximum water holding capacity (MWHC) and stored at 20°C in darkness for seven days before use. Physicochemical characteristics of the test soil and water are summarised in Table 7.1.1.2-1.

Table 7.1.1.2-1: Soil characteristics

Soil designation		**Li 10 (12/1680/01)**
Origin		Limburgerhof, RP, Germany
USDA Particle size distribution [%]		
Clay	<0.002 mm	4.3
Silt	0.002 - 0.050 mm	10.0
Sand	0.050 - 2mm	85.8
soil class		loamy sand
DIN Particle size distribution [%]		
Clay	<0.002 mm	4.3
Silt	0.002 - 0.063 mm	10.7
Sand	0.063 - 2mm	85.0
Soil class		silty sand
Organic C [%]		0.88
Organic matter [%]*		1.52
pH ($CaCl_2$)		6.6
pH (H_2O)		7.3
Cation exchange capacity [$cmol^+/kg$]		5.8
Maximum water holding capacity [g/100g dry soil]		26.8
Bulk density [kg/L]		1.311
Microbial biomass [mg C/100g dry soil]		26.9

RP= Rhineland-Palatinate *calculated: organic matter = organic carbon x 1.724

B. STUDY DESIGN

1. Experimental conditions

The pre-incubated soil was treated at a nominal concentration of 5.3 mg ^{14}C-labelled iprodione /kg dry soil, which corresponds to a field application rate of 2000 g a.s./ha, calculated on the basis of an equal distribution in the top 2.5 cm soil layer and a soil density of 1.5 g/cm³. The actual application rate (TAR = total applied radioactivity) was 5.17075 mg/kg (calculated as the mean value of the sum of extractable radioactive residues and non-extractable radioactive residues of both replicates at day 0).

After stirring, soil portions of 50.0 g dry soil equivalents (55.36 g of moist soil at 40% MWHC) were filled into 25 test vessels (23 glass bottles and 2 centrifuge bottles for the 0 DAT samples). This number of vessels was sufficient to allow duplicate sampling at each sampling time and to keep a limited number of reserve vessels. One of the vessels was used to check the homogeneous distribution of the test item in the soil.

The day 0 samples contained in the centrifuge bottles were not incubated but worked up immediately. The remaining 22 test vessels (glass bottles) were sealed with caps, which were equipped with gas inlet and outlet tubes, and were connected in two series (replicate 1 and replicate 2) each via these inlet and outlet tubes. Each series of vessels ended up in a trapping system for collection of CO_2 (0.5 N NaOH) and volatile organics (ethylene glycol, 0.5 M H_2SO_4) during the aerobic phase of the incubation. Throughout the incubation period the samples were continuously aerated with a slight stream of moistened and CO_2 free air.

The treated soil samples were converted to anaerobic conditions after 15 days after treatment (DAT) by flooding the soil with 75 mL high-purity water and changing the flow-through gas to humidified nitrogen. All incubation vessels were kept in the dark at 20 ± 2ºC. The last samples were taken at 119 DAT after 104 days of anaerobic incubation. To monitor the conversion of the soil from aerobic to anaerobic conditions, the redox potential in soil and water phase, as well as the pH value and O_2-content in the water phase were measured in replicate 2 of each sample during the anaerobic incubation period.

2. Sampling

Aerobic Incubation: Two replicate samples (replicate 1 and replicate 2) were taken for workup and analysis at 0 and 14 days after treatment (DAT) during the aerobic phase.

Anaerobic Incubation: Two replicates samples (replicate 1 and replicate 2) were taken for workup and analysis at 4, 11, 26, 40, 55, 75 and 104 after flooding (DAF), which corresponded to 19, 26, 41, 55, 70, 90 and 119 days after treatment (DAT), respectively. At each sampling time, replicate 1 was worked-up and analysed immediately, whereas replicate 2 was first used for physical measurements (redox potential, pH value and O_2-content) and afterwards, analysed as described below.

The solutions in the traps for volatiles were collected at each sampling time and replaced with fresh solutions, except for the 0 DAT samples.

The soil samples contained in the centrifuge bottles were not incubated but extracted immediately as the day 0 samples.

3. Description of analytical procedures

Soil samples incubated under aerobic conditions were extracted three times with acetonitrile and three times with acetonitrile/water (50:50; v/v) for about 20 minutes each, followed by two additional extraction steps with acetonitrile/water (25:75; v/v) conducted overnight each time, except for 0 DAT samples that received only six extractions á 20 minutes each. Water-logged soil samples incubated under anaerobic conditions were separated from the water phase and extracted as described for the aerobic samples. The individual extracts and water layers were analysed by liquid scintillation counting (LSC). The water layer and the combined acetonitrile extracts as well as the combined acetonitrile/water extracts were analysed by radio-HPLC.

After extraction, the remaining soil was combusted in order to determine the amount of non-extractable radioactive residues (NER, bound residues), which were further characterised by NaOH extraction and subsequent separation into humic and fulvic acid fractions. The fulvic acid fraction was further characterised by partitioning with ethyl acetate.

Identification of the parent compound and its transformation products was achieved by chromatographic comparison with the retention time of reference items. The structures of iprodione and its metabolites were additionally confirmed by means of mass spectrometry (LC-MS/MS) from a number of representative soil extracts

4. Calculation of the degradation rate

Kinetic analysis and calculations of DT_{50} and DT_{90} values for iprodione was performed following the recommendations of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. Degradation products occurring during the study period were not considered in the kinetic evaluation. The analysis was done by non-linear regression methods using the software package KinGUI version 1.2. Parameter estimation was performed considering both the aerobic and the anaerobic phase of the study (1st and 2nd phase, respectively).

II. RESULTS AND DISCUSSION

A.TEST CONDITIONS

Moderately reducing conditions in the water-logged soil layers were established already 4 days after flooding (DAF). Anaerobic conditions were maintained and further developed until the end of the study. At 40-55 DAF, highly anaerobic conditions were reached.

B. MASS BALANCE

The material balance ranged from 90.2% to 100.0% TAR considering the mean values of all sampling times, with an average value of 94.7% TAR.

C. EXTRACTABLE AND BOUND RESIDUES

The distribution of radioactivity in extractable residues (EER), non-extractable residues (NER), CO_2, and other volatiles is shown in Table 7.1.1.2-2.

The amount of extractable radioactivity (ERR) decreased markedly from an average of 98.8% of the total applied radioactivity (TAR) at 0 DAT to 58.4% TAR at 14 DAT (aerobic incubation period) and dropped down to 34.2% TAR at 119 DAT (anaerobic incubation). ^{14}C-residues in the water layer after flooding represented the smallest portion never exceeding 6.8% TAR. For all samples, the number and kind of extraction steps with either acetonitrile or acetonitrile/water was sufficient to capture all the extractable residues. Substantial amounts of non-extractable residues (NER) were formed during the course of the study reaching 37.5% TAR after 14 days of aerobic incubation. Under anaerobic conditions, NER values slowly increased to a maximum of 57.8% TAR at 70 DAT further decreasing to 53.6% by the end of the study. Characterisation of the non-extractable radioactive residues revealed that the predominant part of the radioactivity was tightly bound to the humins or incorporated into humic acids. Since iprodione was detectable only in trace amounts in the NER, it is assumed that no significant amounts of iprodione can be released from the bound residues, even with harsh and artificial extraction methods.

Table 7.1.1.2-2: Distribution of radioactivity and material balance in Li 10 soil after application of ^{14}C-iprodione following 15 days of aerobic and 104 days of anaerobic incubation [% TAR]

DAT	ERR				NER	Volatiles *				Material balance
	ACN	ACN + water	Water layer	Total		CO_2	Ethylene glycol trap	H_2SO_4 trap	Total	
0 (Rep 1)	95.9	2.5	n.a	98.4	1.2	n.a	n.a	n.a	n.a	99.7
0 (Rep 2)	96.4	2.8	n.a	99.1	1.2	n.a	n.a	n.a	n.a	100.3
0 (mean)	**96.2**	**2.6**	**n.a**	**98.8**	**1.2**	**n.a**	**n.a**	**n.a**	**n.a**	**100.0**
14 (rep 1)	43.4	14.8	n.a	58.2	38.6	0.6	0.0	0.0	0.6	97.3
14 (rep 2)	43.7	15.0	n.a	58.7	36.4	1.0	0.0	0.0	1.0	96.2
14 (mean)	**43.6**	**14.9**	**n.a**	**58.4**	**37.5**	**0.8**	**0.0**	**0.0**	**0.8**	**96.8**
19 (rep 1)	37.9	10.1	6.1	54.2	43.8	0.7	0.0	0.0	0.7	98.6
19 (rep 2)	37.4	10.3	6.0	53.6	39.9	1.1	0.0	0.0	1.2	94.7
19 (mean)	**37.6**	**10.2**	**6.1**	**53.9**	**41.8**	**0.9**	**0.0**	**0.0**	**0.9**	**96.7**
26 (rep 1)	34.4	10.0	6.8	51.2	44.4	0.7	0.0	0.0	0.7	96.3
26 (rep 2)	33.2	10.2	6.7	50.1	43.5	1.2	0.0	0.0	1.2	94.8
26 (mean)	**33.8**	**10.1**	**6.8**	**50.6**	**43.9**	**0.9**	**0.0**	**0.0**	**1.0**	**95.5**
41 (rep 1)	30.8	9.6	6.2	46.6	44.4	0.8	0.1	0.0	0.9	92.0
41 (rep 2)	28.5	9.8	6.1	44.4	44.2	1.3	0.0	0.0	1.3	89.9
41 (mean)	**29.7**	**9.7**	**6.2**	**45.5**	**44.3**	**1.0**	**0.1**	**0.0**	**1.1**	**91.0**
55 (rep 1)	27.5	8.7	5.9	42.1	48.1	0.9	0.2	0.0	1.1	91.3
55 (rep 2)	25.7	8.7	5.3	39.7	48.2	1.4	0.4	0.0	1.8	89.6
55 (mean)	**26.6**	**8.7**	**5.6**	**40.9**	**48.1**	**1.2**	**0.3**	**0.0**	**1.4**	**90.5**
70 (rep 1)	24.3	9.0	4.9	38.1	59.9	1.0	0.3	0.0	1.3	99.4
70 (rep 2)	24.1	9.2	5.1	38.3	55.8	1.5	0.8	0.0	2.3	96.4
70 (mean)	**24.2**	**9.1**	**5.0**	**38.2**	**57.8**	**1.3**	**0.5**	**0.0**	**1.8**	**97.9**
90 (rep 1)	23.4	8.0	4.6	36.0	52.6	1.1	0.4	0.0	1.5	90.1
90 (rep 2)	22.3	8.5	4.5	35.3	59.4	1.6	1.1	0.0	2.8	97.4
90 (mean)	**22.9**	**8.2**	**4.6**	**35.6**	**56.0**	**1.4**	**0.7**	**0.0**	**2.1**	**93.7**
119 (rep 1)	23.5	7.9	3.9	35.3	48.1	1.2	0.5	0.0	1.7	85.1
119 (rep 2)	20.7	8.6	3.7	33.0	59.0	1.7	1.5	0.1	3.2	95.3
119 (mean)	**22.1**	**8.3**	**3.8**	**34.2**	**53.6**	**1.4**	**1.0**	**0.0**	**2.5**	**90.2**

n.a. = not analysed, ERR = extractable residues, NER = non-extractable residues

C. VOLATILISATION

Mineralisation was very low throughout the study with CO_2 amounts reaching 0.8% TAR after 14 days of aerobic incubation. During the anaerobic phase from 15 to 119 DAT, the mineralisation rate increased only marginally to 1.4% TAR by the end of the study. No other volatile compounds were detected in significant amounts throughout the incubation period of 119 days.

E. TRANSFORMATION OF PARENT COMPOUND

Results from the radio-HPLC analysis of the soil extracts after treatment of Li 10 soil with ^{14}C-iprodione following 15 days of aerobic and 104 days of anaerobic incubation are presented in Table 7.1.1.2-3. Concentrations of iprodione in soil decreased fast to an average of 12.8% TAR after 14 days of aerobic incubation. After switching from aerobic to anaerobic conditions, a slower degradation was observed with iprodione amounts decreasing to 3.0% TAR by the end of the study.

A number of metabolites was formed during the course of the study, but only two of them, Reg.No. 85831 (RP 32596) and Reg.No. 5079647 (RP 30228), reached concentrations > 5% TAR. Both metabolites were already formed during the aerobic incubation and were also present during the anaerobic phase. Reg.No. 85831 was detected at maximum amounts of 29.8% TAR (41 DAT) slowly decreasing to 20.5% TAR by 119 DAT. Reg.No. 5079647 was formed during the aerobic phase at a maximum of 6.6% TAR with little changes in the concentration (4.5 to 7.5% TAR) during the anaerobic incubation until the end of the study.

Three further degradation products were identified that were formed only in minor amounts < 5% TAR including Reg.No. 207099 (RP 25040), Reg.No. 5079626 (RP 35606) and Reg.No. 5079618 (RP 36221). All three compounds were already formed during the aerobic phase in amounts of 4.3% (Reg.No. 207099), 1.4% (Reg.No. 5079626), and 1.0% TAR (Reg.No.5079618). Concentrations of Reg.No. 207099 and Reg.No. 5079626 decreased during the anaerobic phase by the end of the study to 0.1% and 0.0% TAR, respectively, while concentrations of Reg.No. 5079618 remained at a rather constant level between 0.7 and 1.3% TAR.

All other peaks appeared only sporadically and in negligible amounts and none of the individual components exceeded 2.3% TAR at any sampling time.

Table 7.1.1.2-3: Radio-HPLC analysis of the soil extracts after treatment of Li 10 soil with ^{14}C-iprodione following 15 days of aerobic and 104 days of anaerobic incubation [% TAR, mean of two replicates]

DAT	207099 t_R ~ 22.4	85831 t_R ~ 27.9	BAS610F t_R ~33.1	5079626 t_R ~ 35.2	5079618 t_R ~ 36.9	5079647 t_R ~ 38.8	Sum others*	Total
Acetonitrile extracts								
0	-	-	89.7	1.1	0.1	0.2	5.0	96.2
14	3.2	23.6	10.2	0.8	0.7	3.9	1.1	43.6
19	2.7	20.3	9.1	0.8	0.6	3.3	0.9	37.6
26	1.3	21.5	3.9	0.4	0.8	4.7	1.0	33.8
41	0.6	19.2	2.8	0.1	0.7	5.6	0.8	29.7
55	0.3	16.2	2.4	0.2	0.9	5.6	1.0	26.6
70	0.4	9.8	2.5	0.1	1.1	6.6	3.7	24.2
90	0.2	13.4	1.7	0.0	0.8	6.0	0.8	22.9
119	-	12.2	1.8	-	1.0	6.4	0.7	22.1
Water+acetonitrile extracts								
0	-	-	1.7	0.3	0.0	0.0	0.6	2.6
14	1.1	5.0	2.6	0.6	0.3	2.7	2.6	14.9
19	0.8	5.6	1.3	-	0.1	0.8	1.6	10.2
26	0.9	3.0	1.7	-	0.2	0.7	3.6	10.1
41	0.3	6.7	0.7	0.1	0.2	0.6	1.2	9.7
55	0.2	5.3	0.7	0.0	0.2	0.5	1.7	8.7
70	0.1	3.3	0.6	0.1	0.2	0.5	4.4	9.1
90	0.3	3.9	0.6	-	0.2	0.8	2.5	8.2
119	0.1	6.0	1.0	-	0.2	0.5	0.4	8.3
Water phase								
0	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-
19	1.1	3.3	0.3	0.1	-	0.4	0.9	6.1
26	0.4	4.0	0.4	0.0	-	0.6	1.3	6.8
41	0.0	4.0	0.6	0.1	0.0	0.4	1.0	6.2
55	-	3.7	0.3	0.2	0.0	0.4	0.9	5.6
70	0.0	3.4	0.3	0.1	0.0	0.4	0.8	5.0
90	-	3.1	0.2	0.0	-	0.3	0.8	4.6
119	-	2.2	0.2	-	-	0.3	1.0	3.8
Total extracts								
0	-	-	91.4	1.4	0.2	0.2	5.6	98.8
14	4.3	28.6	12.8	1.4	1.0	6.6	3.7	58.4
19	4.6	29.1	10.7	0.8	0.7	4.5	3.4	53.9
26	2.6	28.5	6.0	0.5	1.0	6.0	5.9	50.6
41	0.9	29.8	4.1	0.2	0.9	6.6	3.0	45.5
55	0.5	25.2	3.4	0.5	1.1	6.5	3.6	40.9
70	0.5	16.5	3.3	0.3	1.3	7.5	8.9	38.2
90	0.5	20.4	2.5	0.1	1.0	7.1	4.1	35.6
119	0.1	20.5	3.0	-	1.1	7.3	2.2	34.2

TAR = total applied radioactivity (100% = 5.17075 mg/kg)
DAT = days after treatment
t_R = retention time [min]
rep = replicate
mean = mean value of replicates
* = each single peak < 2.3% TAR
- = no peak detected

Characterisation of non-extractable residues

The non-extractable ^{14}C-residues (NER) in shown in were further characterised by NaOH-extraction and subsequent fractionation into fulvic acids, humic acids and humins. Results are presented in Table 7.1.1.2-4.

Table 7.1.1.2-4: Characterisation of the non-extractable residues (NER) in Li10 soil after application of ^{14}C-iprodione following 15 days of aerobic and 104 days of anaerobic incubation [% TAR]

DAT	NER	NaOH	Fulvic acids			Humic acids	Humins
			Total	Aqueous phase	Ethyl acetate		
14 (rep 1)	38.6	22.5	8.7	2.5	4.6	13.3	16.4
14 (rep 2)	36.4	21.7	8.7	2.5	4.4	12.8	16.4
14 (mean)	**37.5**	**22.1**	**8.7**	**2.5**	**4.5**	**13.1**	**16.4**
19 (rep 1)	43.8	23.7	9.3	2.6	5.2	13.9	17.8
19 (rep 2)	39.9	22.9	9.1	2.5	5.1	13.3	16.8
19 (mean)	**41.8**	**23.3**	**9.2**	**2.6**	**5.1**	**13.6**	**17.3**
26 (rep 1)	44.4	24.1	9.5	*	*	14.7	19.8
26 (rep 2)	43.5	25.9	9.8	2.6	5.0	15.5	19.9
26 (mean)	**43.9**	**25.0**	**9.7**	**2.6**	**5.0**	**15.1**	**19.8**
41 (rep 1)	44.4	23.2	8.6	2.3	4.2	14.5	18.9
41 (rep 2)	44.2	28.5	9.7	2.7	5.3	17.6	22.4
41 (mean)	**44.3**	**25.8**	**9.1**	**2.5**	**4.7**	**16.1**	**20.7**
55 (rep 1)	48.1	26.8	9.2	2.4	5.1	17.2	23.6
55 (rep 2)	48.2	25.8	9.0	2.4	5.1	16.3	20.7
55 (mean)	**48.1**	**26.3**	**9.1**	**2.4**	**5.1**	**16.8**	**22.2**
70 (rep 1)	59.9	28.3	8.6	2.6	5.2	18.8	25.1
70 (rep 2)	55.8	28.0	8.7	2.3	5.5	18.7	25.6
70 (mean)	**57.8**	**28.1**	**8.6**	**2.5**	**5.4**	**18.8**	**25.4**
90 (rep 1)	52.6	29.7	8.9	2.9	5.4	20.8	27.6
90 (rep 2)	59.4	31.0	9.3	3.2	6.0	21.7	29.4
90 (mean)	**56.0**	**30.4**	**9.1**	**3.0**	**5.7**	**21.3**	**28.5**
119 (rep 1)	48.1	23.2	7.7	2.3	4.9	14.6	21.5
119 (rep 2)	59.0	25.6	8.1	2.6	5.0	16.5	22.3
119 (mean)	**53.6**	**24.4**	**7.9**	**2.4**	**4.9**	**15.6**	**21.9**

TAR = total applied radioactivity (100% = 5.17075 mg/kg)
DAT = days after treatment
NER = non-extractable radioactive residues
rep = replicate
mean = mean value of replicates
* sample 26 rep1 was lost during partition

The calculation of the DT_{50} and DT_{90} values was performed following the recommendations of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. The analysis was done using the software package KinGUI version 1.2.

Although determined degradation rates were significantly different from 0, due to the small number of data points available for the initial aerobic incubation phase, it was not considered reasonable to calculate DT_{50}/DT_{90} values for this phase. Therefore, the DT_{50}/DT_{90} endpoints were calculated only for the second incubation phase (anaerobic phase).

Table 7.1.1.2-5: Li10 soil - data for kinetic evaluation

Day	Iprodione [% TAR]
0	99.7*
0	100.3*
14	12.7
14	12.8
19	10.9
19	10.5
26	5.8
26	6.3
41	4.2
41	3.9
55	3.6
55	3.2
70	3.3
70	3.4
90	2.5
90	2.4
119	2.9
119	3.1

TAR Total Applied Radioactivity
* set to material balance at DAT 0 according to FOCUS [*FOCUS (2006)*]

The best model fit was achieved using the Hockey-Stick model. The DT_{50} and DT_{90} values of iprodione were 32.24 and 107.10 days, respectively, in the total system during the anaerobic phase.

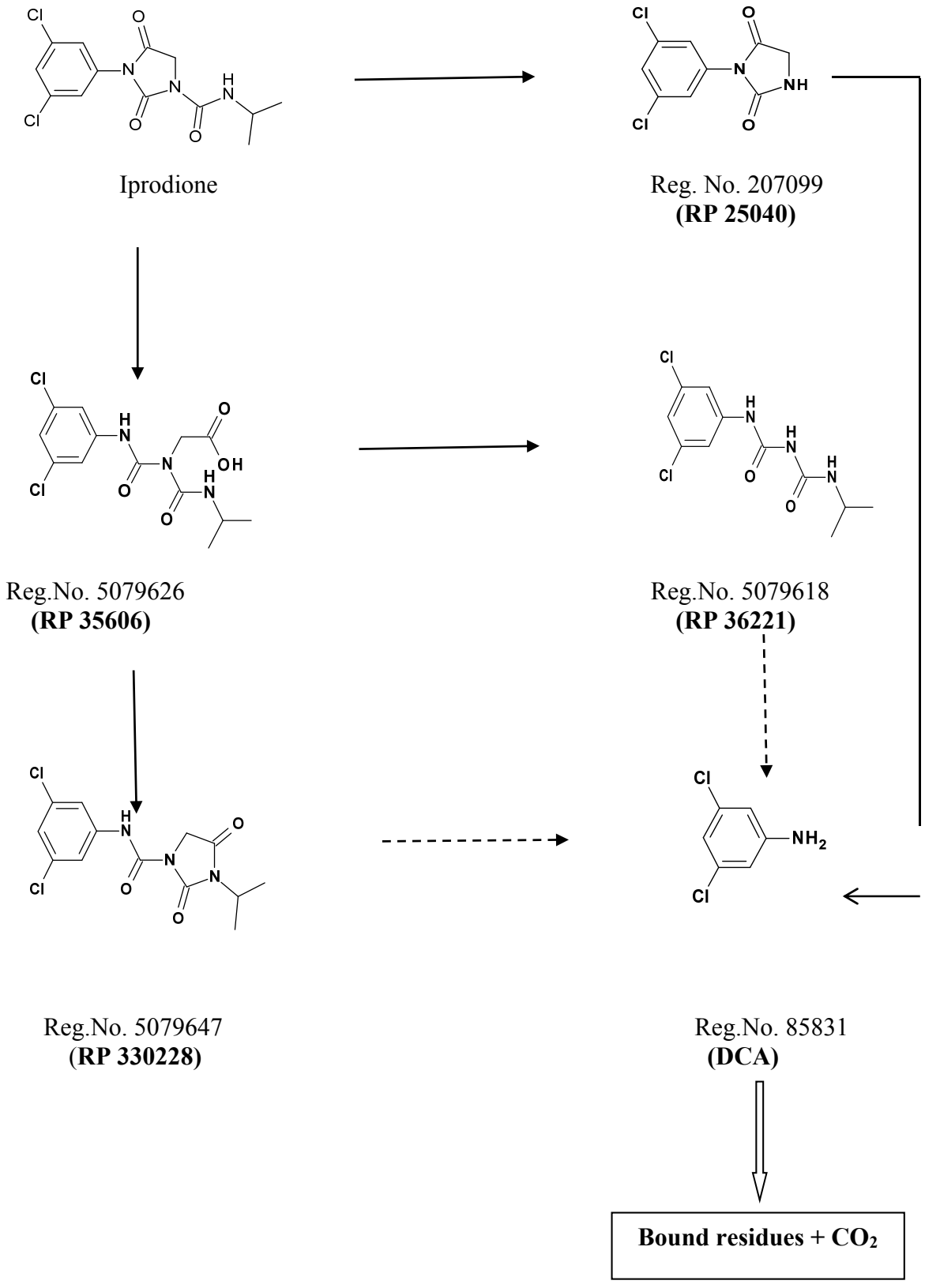
III. CONCLUSION

From the results of the present study it can be concluded that iprodione (BAS 610 F) readily degraded in water-logged soil when incubated in the dark under anaerobic conditions at a temperature of 20°C.

Two metabolites reached concentrations >5% of the total applied radioactivity, which were identified as Reg.No. 85831 (M610F012, RP 32596) and Reg.No. 5079647 (M610F001, RP 30228). Both metabolites were already formed during the aerobic phase of the study.

The results of the study indicate that anaerobic degradation of iprodione basically follows the same course as that followed in aerobic degradation. Best-fit DT_{50} and DT_{90} values for iprodione were 32.24 and 107.10 days, respectively.

Figure 7.1.1.2-1: Proposed route of degradation of iprodione in soil after 15 days of aerobic incubation and 104 days of anaerobic incubation



Supplementary information - Study presented in the original Annex II Dossier (1995):

In a study Gouot [*Gouot (1976) – C022418*] compared aerobic and anaerobic degradation of iprodione. The study showed similar results under aerobic and anaerobic conditions. This would support the current study that both metabolic pathways are basically the same. A short summary of the study can be found below.

Report: Gouot, J.M. et al. (1976): Degradation of RP 26019 in the soil. 2 and 5 ppm treatments with ^{14}C-labelled product.
Report Rhône-Poulenc RP/RD/CNG no 18 785 of August 31,1976.
C022418
GLP: No

A study was conducted in the laboratory with ^{14}C-phenyl labelled iprodione on a loamy soil with an organic matter content of 4.1% and a pH of 7.3. A sample of this soil was treated with iprodione at 5 mg/kg and was then incubated at 23 - 25°C under aerobic conditions for a period of 33 days. After this time it was divided into three lots; one was used for analysis of degradation products; one was returned to aerobic incubation conditions; one was placed into anaerobic incubation conditions. Anaerobic conditions were achieved by placing of the sample in a hermetically sealed beaker which was purged with nitrogen just before being sealed. The incubations continued for a further 111 days (144 days after treatment).

Results: The major degradates in both the anaerobic experiment and the aerobic control were RP 30228 and RP 36221. These were produced in similar quantities in both experiments as is shown in the table below.

Table 7.1.1.2-6: Soil residues in the anaerobic experiment and the aerobic control [% TAR]

Days after treatment.	66		96		144	
Type of incubation	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic
Compound:						
Iprodione (RP 26019)	39.9	41.1	25.4	30.0	15.0	19.1
RP 30228	44.8	44.7	53.9	53.0	49.0	49.6
RP 36221	3.3	2.8	4.0	3.1	4.5	4.5

Bound residues rose to 16% of applied radioactivity in the anaerobic experiment and to 24% in the aerobic control. The half-life in the anaerobic phase (days 33 - 144) was slightly longer in the anaerobic incubation (in which it was 66 days) than in the aerobic control over the same period (in which it was 54 days).

The kinetic analysis of this study was repeated by Szegedi [*Szegedi (2014) – 2013/1311394, CA 7.1.2.1.1/3*] considering latest guidance documents. The determined half-life (trigger $DegT_{50}$) is 38.4 d for the 2 ppm and 39.3 d for the 5ppm system. Details are given in chapter 7.1.2, CA 7.1.2.1.1./2.

CA 7.1.1.3 Soil photolysis

Studies presented in the original Annex II Dossier (1995):
Three studies were performed with iprodione during its registration history. An overview is given in Table 7.1.1.3-1. Except for the study of John et al. [*John et al (1993) – R000277*] they were no longer considered as relevant. The study of John et al. is summarized in this supplementary dossier.

Table 7.1.1.3-1: List of photolysis studies in soil performed with iprodione

Reference	Soil	Application rate [kg/ha]	Wavelength [nm]	Incubation period [days]	Remark
Laurent et. al, 1977 (C022509)	Clay loam	1.9	> 280	31	Not considered relevant
Ayliffe et al. 1991 (C022651)	Sand	5	> 290 < 800	30	Not considered relevant
John et al., 1993 (R000277)	Sandy loam	10		30	Summary

Report: John, A.E. et al. (1993): ^{14}C-Iprodione: Soil photolysis.
Report Rhône-Poulenc Agriculture P93/073 of July 15 1993.
R000277
GLP: Yes

This study to determine the effect of photolysis on the environmental fate of iprodione was conducted on a sandy loam soil. The soil had an organic matter content of 1.3%, a pH of 6.9 and a cation exchange capacity of 12.4 meq/100g.

Table 7.1.1.3-2: Characteristics of test soil

Composition	Sandy loam (USDA)
Sand (0.05 - 2.0 mm) [%]	76
Silt (2 - 50 µ) [%]	11
Clay (< 2 µ) [%]	13
Organic matter [%]	1.34
pH (H_2O)	6.92
CEC [meq/100g]	12.40

The soil was surface treated at a rate equivalent to 10 kg/ha and was incubated at 25 $\pm$ 1°C at 75% of its 1/3 bar moisture holding capacity under aerobic conditions. Test samples were exposed to an artificial light source to simulate summer sunlight in the USA while control samples were kept in the dark. Traps for volatile compounds and carbon dioxide were included in the test systems. The incubation period was 30 days and samples were removed for analysis at various intervals up to this time.

Results: Major metabolites (those which represent > 10% of the applied dose) in the control samples were identified as RP 32596 (3,5-dichloroaniline) and RP 30228, whilst the major metabolites in the photolysis samples were identified as RP 32596 and a mixture of RP 25040 and LS 70942 which could not be separated by the methods used in this study. This mixture represented approximately 14% of applied dose after 7 days photolysis.

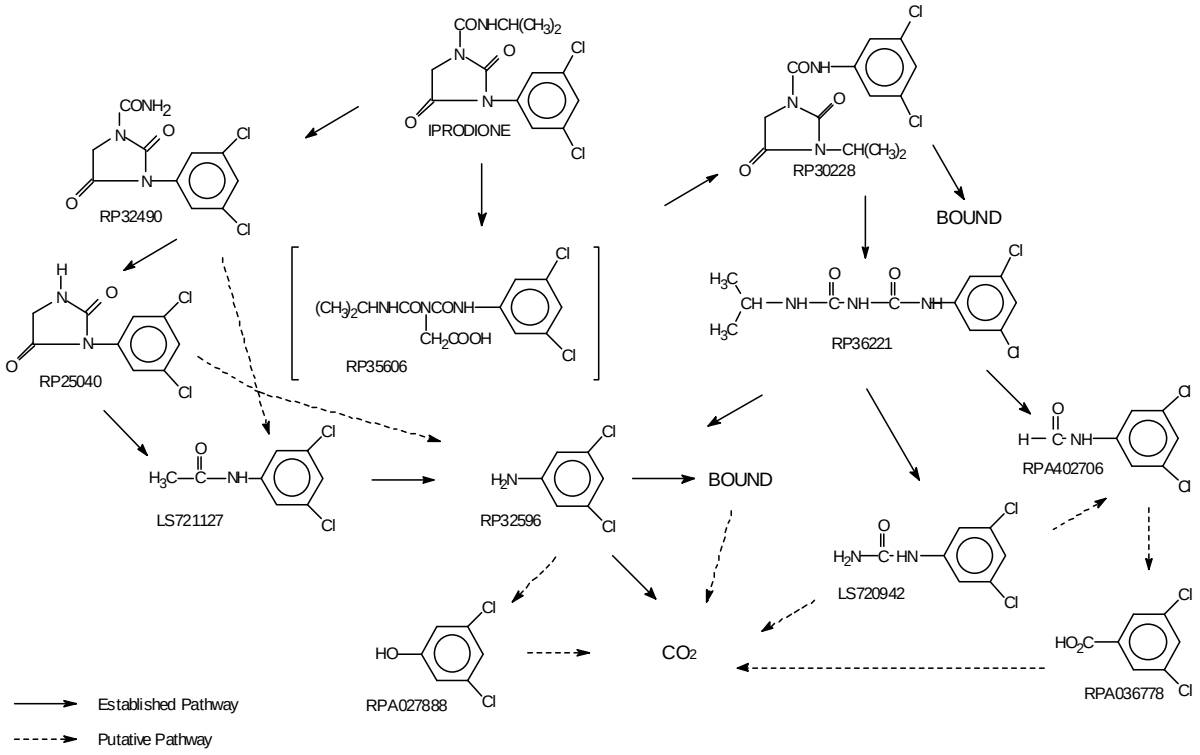
Other metabolites identified in both samples included RP 36221, RP 23490, RPA 402706, LS 721127 and RPA 027888. Most of the metabolites identified were detected in both the control and photolysis samples, the exceptions being RPA 036778 which only appeared in the photolysis samples and RP 32490 which only appeared in the control samples. The common metabolites did, however, occur in different proportions in the two sets of samples.

A higher proportion of carbon dioxide was observed in the photolysis samples (23%) than in control samples (2%). A high degree of binding to the soil (approx. 50%) was observed in both samples. Degradation of iprodione was observed after an initial lag period and half-life values of 5.2 and 4.6 days were calculated for the control and photolysis samples respectively. Similar metabolic profiles were observed for control and photolysis samples.

The original conclusion from this study was that iprodione has a short half-life in soil and that the exposure of the compound to artificial sunlight did not significantly reduce it (5.2 days control vs. 4.6 days irradiated). Close examination of the results show that there was some effect on disappearance of the parent compound because after 7 days the extractable iprodione represented about 97% of that applied in the dark controls and only 67% in the irradiated samples. At 14 days the difference was greater with the figures being 65% and 11% respectively. After this time the disappearance of parent in the dark controls was rapid and by the end of the study the amount of iprodione in controls and irradiated samples was similar.

The conclusions to be drawn from this experiment are that while photolysis is not likely to play a significant role in the rate of disappearance of iprodione over a period of time similar to the incubation period used for this study it may accelerate the initial rate and that whilst the same major degradates will be produced there may be some difference in the proportions of these.

Figure 7.1.1.3-1: Proposed degradation pathway for iprodione in soil in the presence of light (under aerobic conditions)



CA 7.1.2 Rate of degradation in soil

Studies presented in the original Annex II Dossier (1995):
Many studies were performed with iprodione during its registration history. An overview is given in Table 7.1.2.1-1. Except for the studies of Waring [*Waring (1993a, 1993b) – C022365, B003659*] they were no longer considered as relevant, due to high overdosing, insufficient duration or no longer state of the art identification technique. The studies of Waring, are summarized in this supplementary dossier to provide a complete view on the fate of iprodione in the environment.

CA 7.1.2.1 Laboratory studies

Table 7.1.2.1-1: Summary of already evaluated iprodione degradation studies in soils

Reference	Soil	Application rate [mg/kg]	Temperature	Incubation period [days]	Remark
Gouot et al., 1976	Loamy	2 5	23-25 °C 23-25 °C	387 382	Not considered relevant
Gouot et al., 1977	Clay loam Silty clay	1 10 10 10	25°C 25°C 15°C 25°C	385 385 385 385	Not considered relevant
Spare and Po Yung Lu 1978	Loam Sterilised loam Sandy loam Sterilised sandy loam	40 40 40 40	25°C 25°C 25°C 25°C	30 30 30 30	Not considered relevant
Waring 1993a (C022365)	Sandy loam	10	25°C	276	Summarised
Waring 1993 b (B003659)	Loam	14	25^C	100	Summarised

Report: Waring, A.R. (1993a): (^{14}C)-iprodione: aerobic soil metabolism. Report Hazleton Europe 68/132-1015 of December 20, 1993. C022365
GLP: Yes

Summarised under CA 7.1.1.

Report: Waring, A.R. (1993b): (^{14}C)-iprodione: aerobic soil metabolism. Report Hazleton UK 68/139-1015 of July 12, 1993. B003569 (R014527)
GLP: Yes

Method: ^{14}C iprodione of radiochemical purity of 97.2% was applied at a rate of 14 mg/kg (equivalent to 10 kg/ha) in to a loam soil. This soil had an organic matter content of 3.2% and a pH of 6.8. The experiment was performed over a 100 day period, at 25+/-1 °C under aerobic conditions. Sampling intervals were 0, 7, 14, 30, 50 and 100 days. Duplicate samples were analysed after Soxhlet extraction with acetonitrile. Analysis was performed by HLPC with a G type solid scintillant cell and an absorbance detector set at 240 nm for non-radiolabelled standards. Total radioactivity was determined by LSC.
Result: Recovery of radioactivity was better than 95%. Over the period of the study the amount of parent declined to approximately 7%. The main degradation products were RP 30228 and RP 36221 with up to 31% and 17% of applied radioactivity. The DT_{50} and DT_{90} of iprodione were found to be 26 days and 85 days respectively.

The kinetic analysis of this study was repeated by Szegedi [*Szegedi (2014) – 2013/1311394, CA 7.1.2.1.1/3*] considering latest guidance documents. The determined half-life (trigger $DegT_{50}$) is 16.2d. Details are given in chapter 7.1.2, CA 7.1.2.1.1./2.

CA 7.1.2.1.1 Aerobic degradation of the active substance

Not yet peer-reviewed studies:

Report:	CA 7.1.2.1.1/1 Hartmann M. et al., 2014a Aerobic soil metabolism and rate of degradation of BAS 610 F (Iprodione) in EU soils 2013/1240319
Guidelines:	OECD 307, EPA 835.4100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

The report on the rate of degradation of iprodione in aerobic soil is summarised in CA 7.1.1.1/1. Only the degradation rates for the parent are reported in CA 7.1.1.1/1, while a complete kinetic analysis including the metabolites of iprodione observed in the aerobic degradation study is summarised below (CA 7.1.2.1.1/2).

Report: CA 7.1.2.1.1/2
Budde E., 2014b
Kinetic evaluation of a laboratory soil degradation study with Iprodione according to FOCUS degradation kinetics
2013/1311391
Guidelines: FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011
GLP: no

Executive Summary
The degradation of the fungicide BAS 610 F - iprodione in soil has been investigated under aerobic laboratory conditions in four soils with [phenyl-U-^{14}C]-labelled iprodione [*Hartman et al. (2014a) – 2013/1240319, CA 7.1.2.1.1/1*]. The purpose of this evaluation was to analyse the degradation kinetics observed in the soils, taking into account the current guidance of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*].

The appropriate kinetic models to derive non-normalised trigger and normalised modelling endpoints were identified considering the procedures and kinetic models proposed by FOCUS [*FOCUS (2006)*].

Prior to deriving the modelling endpoints, the $DegT_{50}$ values obtained under different incubation conditions were normalised to the reference moisture of pF2 and the reference temperature of 20°C according to the recommendations of FOCUS [*FOCUS (2006)*].

For iprodione, the kinetic evaluation showed that the SFO model is appropriate to derive trigger endpoints for additional work in one soil, while biphasic kinetic models (DFOP or FOMC) were selected as best-fit models in three soils. The best-fit $DegT_{50}$ values were between 6.3 and 91.1 days, and $DegT_{90}$ values were between 73.7 and 376.9 days. The normalised modelling $DegT_{50}$ values were between 15.9 and 80.7 days.

The degradation of iprodione in soil leads to a number of metabolites, some of them observed only in low concentrations. Five metabolites detected in the aerobic soil degradation study were included in this evaluation: RP 25040, RP 35606, RP 30228, RP 36221 and 3,5-dichloroaniline (DCA). The metabolite DCA may theoretically be formed from three different precursors (RP 25040, RP 30228 and RP 36221). In this kinetic evaluation, elucidation of the relevant pathway concerning DCA was attempted. To obtain statistically significant parameters, different pathways had to be considered in individual soils.

For the metabolites of iprodione, trigger endpoints as well as normalised modelling-$DegT_{50}$ values and corresponding formation fractions could be derived for each metabolite in at least one soil.

I. MATERIAL AND METHODS

The degradation of iprodione in four different soils was analysed taking into account the current guidance of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*].

Kinetic modelling strategy

Kinetic evaluation was performed in order to derive i) degradation parameters that are valid as trigger endpoints as well as ii) appropriate degradation parameters for environmental fate models.

For the parent substance iprodione, 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.

For the metabolites of iprodione, best-fit for the two primary metabolites was deemed crucial to derive accurate parameters for secondary metabolites. This in turn is strongly influenced by the degradation pattern of the parent. In soils where the parent iprodione did not follow SFO kinetics, the best-fit biphasic model was therefore implemented for the parent, while SFO was always used for metabolites. All metabolites were added in one step; all parameters (except M_0 of metabolites, which were set to zero) were free.

The degradation pathway of iprodione to its metabolites RP 25040, RP 35606, RP 30228 and RP 36221 has been determined previously in the aerobic degradation study [*Hartman et al. (2014a) – 2013/1240319, CA 7.1.2.1.1/1*]. To determine the relevant precursors of DCA, the possible formation pathways were tested individually, i.e. three separate model runs were set up where DCA was formed from either RP 25040, RP 30228 or RP 36221 (path 1, 2 or 3, respectively in Figure 7.1.2.1-1). Further, combinations of these three paths were run (path1+2+3, path1+2, path1+3, path2+3).

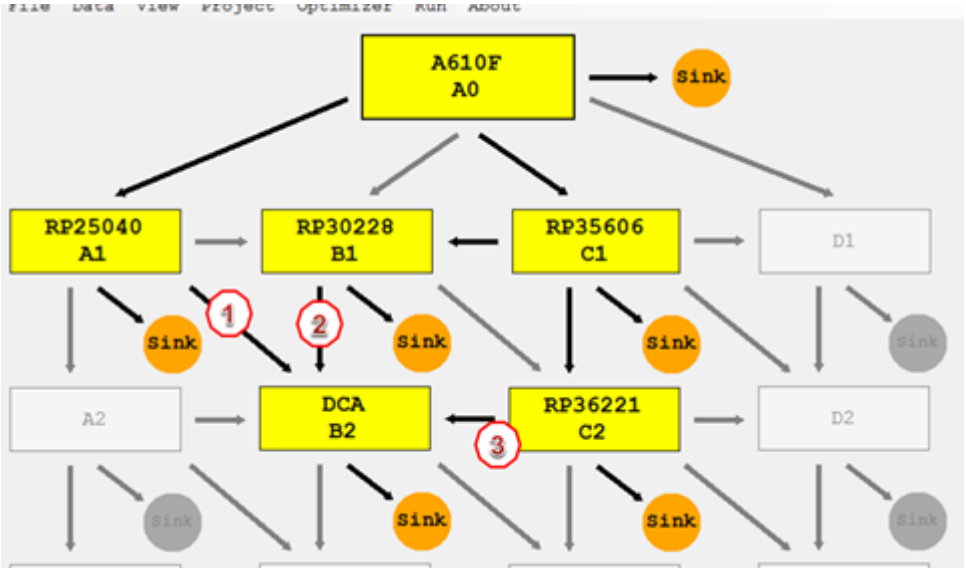


Figure 7.1.2.1-1: Setup of model runs in KinGUI, considering three possible precursors of DCA

Kinetic models included in the evaluations

For each data set, the kinetic models proposed by FOCUS Kinetics [*FOCUS (2006)*] were tested. The recommended kinetic models, i.e. single first order (SFO) kinetics, the Gustafson-Holden model (FOMC), bi-exponential (DFOP) kinetics, and hockey stick (HS) kinetics, were applied. 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 to 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)*].

Due to the number of fit parameters and due to the complex degradation pathway of iprodione, it was unfortunately not possible to gain excellent fits for all substances at the same time. However, the fundamental goal of deriving modelling endpoints is to derive conservative parameters for a subsequent exposure assessment. Considering available sorption degradation and toxicological data on involved substances, metabolites RP 36221 and DCA are of special interest. All other metabolites considered here are either not mobile and/or degrade rapidly and/or are toxicologically not relevant. Accordingly, deviations from the above given criteria were considered as acceptable if the derived parameters were conservative for RP 36221 and DCA.

Data handling and software for kinetic evaluation

When available, replicate measurements were used in the parameter estimation. The experimental data were derived from the study report and adjusted according to FOCUS Kinetics [*FOCUS (2006)*]. As no LOQ was provided, it was conservatively assumed to be 0.1% TAR, and the LOD was set equal to this value.

The software package KinGUI version 2 was used for parameter fitting. The error tolerance and the number of iterations of the optimisation tool were set to 0.00001 and 100, respectively.

Normalisation of degradation rates to reference conditions

The $DegT_{50}$ values were normalised to reference conditions to a temperature of 20°C and a soil moisture at field capacity of pF2 [*FOCUS (2002) "Generic guidance for FOCUS groundwater scenarios", v 1.1*].

The temperature normalisation was performed using the temperature correction factor in Equation 7.1.2.1.1-1, and a default Q_{10} value of 2.58 was considered [*FOCUS (2006) v.1.0 from November 2011*]. The $DegT_{50}$ moisture normalisation was performed using the moisture dependency equations by Walker as described in Equation 7.1.2.1.1-2.

Equation 7.1.2.1.1-1 Temperature correction factor for $DegT_{50}$ values

$$f_{temp} = Q_{10}^{\frac{Tact - Tref}{10}}$$

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)	[-]

Equation 7.1.2.1.1-2 Moisture correction factor for $DegT_{50}$ values according to Walker

$$f_{moist} = \begin{cases} \left(\frac{\theta_{act}}{\theta_{ref}}\right)^{0.7} & if \quad \theta_{act} < \theta_{ref} \\ 1 & if \quad \theta_{act} \geq \theta_{ref} \end{cases}$$

with:			
	f_{moist}	moisture correction factor	[-]
	θ_{ref}	reference soil moisture at field capacity (pF2, 10 kPa)	[g / 100 g dry soil]
	θ_{act}	actual soil moisture during incubation	[g / 100 g dry soil]

The actual soil moisture and the corresponding water content at pF2 for each soil were taken from the study report.

The $DegT_{50}$ values normalised to reference conditions were calculated by multiplying the $DegT_{50}$ values at study conditions by the correction factors f_{temp} and f_{moist} as described in Equation 7.1.2.1.1-3.

Equation 7.1.2.1.1-3 Calculation of the $DegT_{50}$ at reference conditions (20 °C, moisture at pF2)

$$DT_{50\_ref} = DT_{50} \cdot f_{temp} \cdot f_{moist}$$

Experimental data

The kinetic evaluation was based on the findings of a study that can be found in [*Hartman et al. (2014a) – 2013/1240319, CA 7.1.2.1.1/1*]. The complete data set and soil characteristics can be found there. An overview of the study is given in Table 7.1.2.1.1-1.

Table 7.1.2.1.1-1: Overview on soil aerobic degradation study with iprodione

Soil	Soil type	Incubation			Analyte	% of initial at end of study	Study (DocID)
		Moisture	Temp. [°C]	Time [d]			
LUFA 2.2	Loamy sand	40% MWC	20	120	[phenyl-U-^{14}C]-iprodione	44.1	2013/1240319
Li 10	Loamy sand					7.5	
LUFA 2.3	Sandy loam					7.3	
LUFA 5M	Sandy loam					4.5	

II. RESULTS AND DISCUSSION

Iprodione

The derived trigger endpoints for iprodione are summarised in Table 7.1.2.1.1-2. The kinetic evaluation showed that the SFO model is appropriate to derive trigger endpoints for additional work in one soil, while biphasic kinetic models (DFOP or FOMC) were selected as best-fit models in three soils.

Table 7.1.2.1.1-2: Non-normalised trigger endpoints for iprodione based on best-fit models

Soil	Best-fit model	Trigger endpoints	
		$DegT_{50}$ [d]	$DegT_{90}$ [d]
LUFA 2.2	DFOP	91.1	376.9
Li 10	SFO	43.5	144.4
LUFA 2.3	FOMC	9.6	99.2
LUFA 5M	DFOP	6.3	73.7

Modelling endpoints for iprodione could be derived from SFO kinetics in two soils, and from FOMC in the other two soils. The $DegT_{50}$ values suitable for modelling obtained under different incubation conditions were normalised to a reference moisture of pF2 and a temperature of 20°C. Parameters included in the normalisation procedure as well as derived modelling endpoints are summarised in Table 7.1.2.1.1-3.

Table 7.1.2.1.1-3: Normalisation of iprodione degradation rates to standard conditions

Soil	pH ($CaCl_2$)	Kinetic model	T_{act}	T_{ref}	θ_{act}	θ_{ref}	f_{temp}	f_{moist}	$DegT_{50act}$	$DegT_{50ref}$
LUFA 2.2	5.5	SFO	20	20	13.4	16.7	1.0	0.855	94.4	80.7
Li 10	6.2	SFO	20	20	9.3	10.2	1.0	0.936	43.5	40.7
LUFA 2.3	6.7	FOMC	20	20	11.3	16.4	1.0	0.770	29.9	23.0
LUFA 5M	7.3	FOMC	20	20	11.8	22.1	1.0	0.643	24.7	15.9

T_{act}	actual temperature during incubation	[°C]
T_{ref}	reference temperature (20°C)	[°C]
θ_{act}	actual soil moisture (40% of MWC)	[g / 100 g dry soil]
θ_{ref}	reference soil moisture at field capacity (pF 2)	[g / 100 g dry soil]
f_{temp}	temperature correction factor	[-]
f_{moist}	moisture correction factor	[-]
$DegT_{50act}$	$DegT_{50}$ at study conditions	[d]
$DegT_{50ref}$	$DegT_{50}$ at reference conditions	[d]

Metabolites of iprodione

To obtain statistically significant parameters for the metabolites of iprodione, different pathways had to be considered in individual soils. This can be explained by the different amounts of residues that appeared in different soils: where only very small amounts of individual metabolites were observed, no significant parameters could be derived for the respective metabolite.

Soil LUFA 2.2

The results of the model runs with parent (DFOP) and metabolites (all SFO) indicated that the inclusion of RP 36221 as a precursor ('path 3') had negative effects on the χ^2 error of DCA. If RP 30228 was the only precursor ('path 2'), the model fit was also poor for DCA. The most relevant pathway for soil LUFA 2.2 was therefore 'path 1', with RP 25040 as only precursor of DCA.

The metabolites RP 25040 and RP 30228 (possible precursors of DCA) as well as metabolite RP 35606 (precursor of RP 30228 and RP 36221) were not much affected by the pathway chosen for DCA formation.

The complete parameter estimations for this pathway ('**path 1**') are given in the table below.

Table 7.1.2.1.1-4: Parameters for metabolites of iprodione derived from the selected pathway 'path 1' in soil LUFA 2.2

Parameter	RP 25040	RP 36221	RP 30228	RP 35606	DCA
χ^2 [%]	18.1	11.5	12.1	14.2	28.6
k-rate [1/d]	0.0308	0.0038	0.0161	0.2196	0.0184
$DegT_{50}$ [d]	22.5	180.4	43.1	3.2	37.7
$DegT_{90}$ [d]	74.7	599.3	143.1	10.5	125.4
Type I error of k-rate	<0.05	<0.05	<0.05	<0.05	<0.05
ff [-]	0.17	0.15	0.21	0.52	1.00 *
ff sig.	<0.05	<0.05	<0.05	<0.05	<0.05

* from RP 25040

Soil Li10

The results of the model runs with parent (SFO, best-fit) and metabolites (all SFO) indicated that the metabolites RP 25040 and RP 30228 (possible precursors of DCA) as well as metabolite RP 35606 (precursor of RP 30228 and RP 36221) were not much affected by the pathway chosen for DCA formation. Metabolite RP 36221 was usually well fitted, except where it was the only precursor of DCA ('path 3'). DCA was fitted best in any combination with RP 25040 as precursor ('path 1'), even if RP 25040 itself showed a high χ^2 error. This high error can be attributed to a high concentration found in the day 21 sample, which was not simulated well in any of the model runs.

The most relevant pathway for soil Li 10 was therefore '**path 1+2**', with RP 25040 and RP 30228 as precursors of DCA. The complete parameter estimation for this pathway is given in the table below.

Table 7.1.2.1.1-5: Parameters for metabolites of iprodione derived from the selected pathway 'path 1+2' in soil Li 10

Parameter	RP 25040	RP 36221	RP 30228	RP 35606	DCA
χ^2 [%]	37.6	15.7	11.2	22.8	20.8
k-rate [1/d]	0.0579	<0.001	0.0142	0.2133	<0.001
$DegT_{50}$ [d]	12.0	>1000	48.7	3.2	>1000
$DegT_{90}$ [d]	39.8	>1000	161.7	10.8	>1000
Type I error of k-rate	<0.05	<0.05	<0.05	<0.05	not sig.
ff [-]	0.19	0.07	0.28	0.82	0.62 / 0.21 *
ff sig.	<0.05	<0.05	<0.05	<0.05	not sig.

* from RP 25040 / from RP 30228

Soil LUFA 2.3

In the model runs with parent (FOMC, best-fit) and metabolites (all SFO), it could be observed that RP 25040 showed a variation in the peak shape (depending on whether it was a precursor of DCA or not), associated with a varying χ^2 error (ca. 30% / ca. 40%). Runs where iprodione and its first metabolites (RP 25040 and/or RP 35606) were fixed to separately determined parameters (from a run including only iprodione, RP 25040 and RP 35606) yielded worse results for the later metabolites; therefore, the run with all parameters free was kept.

No single path or combinations of paths stands out, and thus all paths ('**path 1+2+3**') were included for derivation of modelling parameters. RP 35606 had its peak early in the study (<10 days) and was not well simulated when all paths were included in the model. However, its degradation products, RP 30228 and RP 36221, were well simulated in that case. The complete parameter estimation for this pathway is given in the table below.

Table 7.1.2.1.1-6: Parameters for metabolites of iprodione derived from the selected pathway 'path 1+2+3' in soil LUFA 2.3

Parameter	RP 25040	RP 36221	RP 30228	RP 35606	DCA
χ^2 [%]	40.6	14.0	9.8	30.6	14.0
k-rate [1/d]	0.1011	<0.001	0.0081	0.2594	0.0172
$DegT_{50}$ [d]	6.9	>1000	86.1	2.7	40.3
$DegT_{90}$ [d]	22.8	>1000	286.0	8.9	133.9
Type I error of k-rate	<0.05	not sig.	<0.05	<0.05	not sig.
ff [-]	0.15	0.08	0.22	0.85	1 / 1 / 0.003 *
ff sig.	<0.05	<0.05	<0.05	<0.05	not sig.

* from RP 25040 / RP 30228 / from RP 36221

Soil LUFA 5M

The results of the model runs with parent (DFOP, best-fit) and metabolites (all SFO) indicated that the metabolites RP 30228 (possible precursor of DCA) as well as metabolite RP 35606 (precursor of RP 30228) were well fitted and not much affected by the pathway chosen for DCA formation. Metabolite RP 36221 was usually well fitted, except where it was the only precursor of DCA ('path 3'). DCA was fitted best in any combination with RP 30228 as precursor ('path 2'), and also if RP 36221 ('path 3') was the only precursor. The metabolite RP 25040 only provided an adequate fit when it was not a precursor of DCA.

Overall, the best results were obtained when RP 30228 was a precursor of DCA ('path 2') and RP 25040 ('path 1') was not. For PEC modelling of the complete pathway, RP 36221 ('path 3') as a precursor should be included for conservative concentration estimations, as this path represents an uncertainty and the derived parameters differ significantly between 'path 2' and 'path 2+3'. The complete parameter estimation for this pathway ('**path 2+3**') is given in the table below.

Table 7.1.2.1.1-7: Parameters for metabolites of iprodione derived from the selected pathway 'path 2+3' in soil LUFA 5M

Parameter	RP 25040	RP 36221	RP 30228	RP 35606	DCA
χ^2 [%]	26.3	13.9	15.5	16.7	12.9
k-rate [1/d]	<0.001	<0.001	0.0306	0.0665	0.0446
$DegT_{50}$ [d]	>1000	>1000	22.7	10.4	15.5
$DegT_{90}$ [d]	>1000	>1000	75.3	34.6	51.7
Type I error of k-rate	not sig.	not sig.	<0.05	<0.05	<0.05
ff [-]	0.01	0.08	0.92	0.76	0.54 / 0.21 *
ff sig.	<0.05	not sig.	<0.05	<0.05	<0.05 / not sig.*

* from RP 30228 / from RP 36221

Summary for the metabolites of iprodione

For the metabolites, the derived SFO-$DegT_{50}$ values are suitable as trigger endpoints and for derivation of modelling endpoints after normalisation to reference conditions. The same correction factors f_{temp} and f_{moist} as for the parent (see Table 7.1.2.1.1-3) were used for calculation of normalised $DegT_{50}$ values (Table 7.1.2.1.1-8).

Table 7.1.2.1.1-8: Normalisation of metabolite $DegT_{50}$ values derived from the selected pathways in the four soils to standard conditions

Soil	pH ($CaCl_2$)	f_{temp}	f_{moist}	$DegT_{50act}$	$DegT_{50ref}$
RP 25040					
LUFA 2.2	5.5	1.0	0.855	22.5	19.2
Li 10	6.2	1.0	0.936	12.0	11.2
LUFA 2.3	6.7	1.0	0.770	6.9	5.3
LUFA 5M	7.3	1.0	0.643	- *	- *
RP 36221					
LUFA 2.2	5.5	1.0	0.855	180.4	154.2
Li 10	6.2	1.0	0.936	- *	- *
LUFA 2.3	6.7	1.0	0.770	- *	- *
LUFA 5M	7.3	1.0	0.643	- *	- *
RP 30228					
LUFA 2.2	5.5	1.0	0.855	43.1	36.8
Li 10	6.2	1.0	0.936	48.7	45.6
LUFA 2.3	6.7	1.0	0.770	86.1	66.3
LUFA 5M	7.3	1.0	0.643	22.7	14.6
RP 35606					
LUFA 2.2	5.5	1.0	0.855	3.2	2.7
Li 10	6.2	1.0	0.936	3.2	3.0
LUFA 2.3	6.7	1.0	0.770	2.7	2.1
LUFA 5M	7.3	1.0	0.643	10.4	6.7
DCA					
LUFA 2.2	5.5	1.0	0.855	37.7	32.2
Li 10	6.2	1.0	0.936	- *	- *
LUFA 2.3	6.7	1.0	0.770	40.3	31.0
LUFA 5M	7.3	1.0	0.643	15.5	10.0

f_{temp}	temperature correction factor	[-]
f_{moist}	moisture correction factor	[-]
$DegT_{50act}$	$DegT_{50}$ at study conditions	[d]
$DegT_{50ref}$	$DegT_{50}$ at reference conditions	[d]
*	no reliable degradation rate could be determined	

An overview of the formation fractions of the metabolites derived from the selected paths is given in Table 7.1.2.1.1-9.

Table 7.1.2.1.1-9: Formation fractions of the metabolites of iprodione derived from the selected pathways in the four soils

Soil	Formation fraction [-]				
	Iprodione ↓ RP 25040	Iprodione ↓ RP 35606	RP 35606 ↓ RP 30228	RP 35606 ↓ RP 36221	RP 25040 / RP 30228 / RP 36221 ↓ DCA
LUFA 2.2	0.17	0.52	0.21	0.15	1 / - / -
Li 10	0.19	0.82	0.28	0.07	- / - / -
LUFA 2.3	0.15	0.85	0.22	0.08	- / - / -
LUFA 5M	0.01	0.76	0.92	-	- / 0.54 / -

- no reliable formation fraction could be determined

III. CONCLUSION

Trigger and modelling endpoints were derived for iprodione in a laboratory degradation study with four soils.
For iprodione, the kinetic evaluation showed that the SFO model is appropriate to derive trigger endpoints for additional work in one soil, while biphasic kinetic models (DFOP or FOMC) were selected as best-fit models in three soils. The best-fit $DegT_{50}$ values were between 6.3 and 91.1 days, and $DegT_{90}$ values were between 73.7 and 376.9 days. The normalised modelling $DegT_{50}$ values were between 15.9 and 80.7 days.

For the metabolites of iprodione, trigger endpoints as well as normalised modelling-$DegT_{50}$ values and corresponding formation fractions could be derived for each metabolite in at least one soil.

Report:	CA 7.1.2.1.1/3 Szegedi K., 2014a Update of the kinetic evaluations of older soil degradation studies with BAS 610 F (Iprodione) and DCA (3,5-Dichloroaniline) according to the FOCUS kinetics guideline 2013/1311394
Guidelines:	FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011
GLP:	no

Executive Summary

The degradation of the fungicide BAS 610 F - iprodione in soil has been investigated under aerobic laboratory conditions in one soil with [phenyl-U-^{14}C]-labeled iprodione at two different initial concentrations [*Gouot et al. (1976) – C022418*]. Additionally, degradation of [phenyl-U-^{14}C]-labeled iprodione was studied in was studied in two soils at one initial concentration per soil [*Waring (1993a) – R014527; Waring (1993b) – C022365*].

The degradation of DCA (3,5-dichloroaniline) in soil has been investigated under aerobic laboratory conditions in two studies in three soils with ^{14}C-labeled 3,5-dichloroaniline [*Gouot (1981) – C022371; Quarmby (2000) – B002966*].

The purpose of this evaluation was to analyze the degradation kinetics observed in the soils, taking into account the current guidance of the FOCUS workgroup on degradation kinetics.

Kinetic evaluation was performed in order to derive
i) Degradation parameters as triggers for additional work (trigger endpoints)
ii) Degradation parameters for environmental fate models (modelling endpoints)

Obtained endpoints are summarised in the conclusions.

I. MATERIAL AND METHODS

The degradation of the fungicide BAS 610 F - iprodione in soil has been investigated under aerobic laboratory conditions in one soil with [phenyl-U-^{14}C]-labeled iprodione at two different initial concentrations [*Gouot et al. (1976) – C022418*]. Additionally, degradation of [phenyl-U-^{14}C]-labeled iprodione was studied in was studied in two soils at one initial concentration per soil [*Waring (1993a) – R014527; Waring (1993b) – C022365*].

The degradation of DCA (3,5-dichloroaniline) in soil has been investigated under aerobic laboratory conditions in two studies in three soils with ^{14}C-labeled 3,5-dichloroaniline [*Gouot (1981) – C022371; Quarmby (2000) – B002966*].

Study conditions and results are available in the respective study reports and briefly summarised in chapter CA 7.1.

Kinetic evaluation

Kinetic analysis and calculations were performed using data obtained in the above given soils and studies. The recommendations of the FOCUS Kinetics workgroup were followed [*FOCUS (2006)*]. Degradation products occurring during the study period were not considered in the kinetic evaluation. The analysis was done by non-linear regression methods using the software package KinGUI version 1.2.

Kinetic models
For the data set, the kinetic models proposed by the FOCUS Kinetics guidance document [*FOCUS (2006)*] were tested in order to identify the best-fit model for the active substance iprodione. The recommended kinetic models, i.e. the single first order kinetics (SFO), the Gustafson-Holden model (FOMC), hockey-stick (HS) and bi-exponential (DFOP) kinetics were applied by using KinGUI. The respective model descriptions and corresponding equations for calculating endpoints (DT_{50}, DT_{90}) are given in FOCUS [*FOCUS (2006)*].

Goodness-of-fit statistics
The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended in FOCUS [*FOCUS (2006)*]. For visual inspection, the recommended graphical representations of observed and modeled decline curves versus time and the residuals versus time are presented.
The goodness-of-fit measure used for identification of the best-fit kinetic model is the χ^2-minimum error level as given in FOCUS [*FOCUS (2006)*]. The kinetic endpoints (DT_{50} and DT_{90} values) are reported for the best-fit model, which is selected based on statistical and visual assessment.

The goodness-of-fit statistics, i.e. χ^2 error level and type-I error rate, were calculated within the KinGUI runs and documented in the respective output files, which are included in the study report.

Data handling
When available, replicate measurements were used in the parameter estimation. The experimental data were adjusted according to FOCUS [*FOCUS (2006)*]. The initial concentration of the applied substance was set to the material balance recovered at day 0. The measured data as well as resulting datasets submitted to kinetic analysis are given in the modelling report.

The χ^2 value for the kinetic model was calculated as recommended by FOCUS [*FOCUS (2006)*], considering the average of replicate measurements. For the individual parameters, the t-test statistics were based on number of individual measurements.

Software for kinetic evaluation
The software package KinGUI (version 1.2) was used for parameter fitting [*Schäfer et al. (2007) – BASF DocID 2007/1062781*]. The error tolerance and the number of iterations of the optimisation tool were set to 0.00001 and 1000, respectively.

II. RESULTS AND DISCUSSION

The datasets for each soil were analysed considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. Only the final results of the evaluation are presented in this section, while the documentation of the evaluation steps, the complete fitting procedure, including all fit parameters, is compiled in the modelling report

An overview of the endpoints of the metabolite iprodione derived from the laboratory studies is given in Table 7.1.2.1.1-10 and in Table 7.1.2.1.1-11.

Table 7.1.2.1.1-10: Trigger endpoints for iprodione based on best-fit models

Soil	Best-fit model	Trigger endpoints	
		$DegT_{50}$ [d]	$DegT_{90}$ [d]
Bondhay [*Waring (1993a) – R014527*]	SFO	16.5	74.7
Sandy loam [*Waring (1993b) – C022365*]	HS	16.2	53.9
Emerainville 2ppm [*Gouot et al. (1976) – C022418*]	DFOP	38.4	198.5
Emerainville 5ppm [*Gouot et al. (1976) – C022418*]	DFOP	39.3	158.2

Table 7.1.2.1.1-11: Modelling endpoints for iprodione

Soil	Kinetic model	Modelling endpoints
		$DegT_{50}$ [d]
Bondhay [*Waring (1993a) – R014527*]	SFO	22.5*
Sandy loam [*Waring (1993b) – C022365*]	HS	16.2
Emerainville 2ppm [*Gouot et al. (1976) – C022418*]	FOMC	56.9*
Emerainville 5ppm [*Gouot et al. (1976) – C022418*]	FOMC	47.7*

* FOMC $DT_{90}/3.32$

Summary of endpoints for DCA
An overview of the endpoints of the metabolite DCA derived from the laboratory studies is given in Table 7.1.2.1.1-12 and in Table 7.1.2.1.1-13.

Table 7.1.2.1.1-12: Trigger endpoints for DCA based on best-fit models

Soil	Best-fit model	Trigger endpoints	
		$DegT_{50}$ [d]	$DegT_{90}$ [d]
'Sandy loam' [*Gouot (1981) – C022371*]	FOMC	8.6	189.9
Columbia / Silt loam [*Quarmby (2000) – B002966*]	FOMC	2.7	74.9
Modera / Sandy loam [*Quarmby (2000) – B002966*]	FOMC	8.6	197.8

Table 7.1.2.1.1-13: Modelling endpoints for DCA

Soil	Kinetic model	Modelling endpoints
		$DegT_{50}$ [d]
'Sandy loam' [*Gouot (1981) – C022371*]	FOMC	57.2*
Columbia / Silt loam [*Quarmby (2000) – B002966*]	FOMC	22.6*
Modera / Sandy loam [*Quarmby (2000) – B002966*]	FOMC	59.6*

* FOMC DT_{90}/3.32

III. CONCLUSION

The derived endpoints are appropriate for use as input for subsequent decisions and / or model calculations.

Overall summary on laboratory degradation rates

An overview of laboratory degradation rates is given in Table 7.1.2.1.1-14.

The following considerations need to be taken into account for the endpoint selection:

Acidic soils:
Studies performed with laboratory soils LUFA 2.2 [*Hartman et al. (2014a) – 2013/1240319, CA 7.1.2.1.1/1*], sandy loam [*Waring (1993a) – R014527*], and the field site Maine-et-Loire [*Richter & Kuhnke (2011) – 2011/1284995, CA 7.1.2.2.1/3*] provide sufficient information on the behaviour of iprodione in acidic soils. Worst-case $DegT_{50}$ determined in soil LUFA 2.2 [*Hartman et al. (2014a) – 2013/1240319, CA 7.1.2.1.1/1; Budde (2013) – 2013/1611391, CA 7.1.2.1.1/2*] will be used for subsequent calculations.

Alkaline soils:
$DegT_{50}$ determined in field studies will be used for subsequent calculations. $DegT_{50}$ determined in laboratory studies shall be considered as additional information.

Table 7.1.2.1.1-14: Degradation of iprodione in soil under aerobic conditions in the laboratory

Soil	Soil type (USDA)	pH_{CaCl2} [-]	Best-fit model	Trigger-$DegT_{50}$ [d]	Trigger-$DegT_{90}$ [d]	Modelling-$DegT_{50}$ [d]	Study / Kinetic evaluation
LUFA 2.2	Loamy sand	5.5	DFOP	91.1	376.9	80.7	Hartman et al. (2013/1240319), Budde (2013/1611391)
Li 10	Loamy sand	6.2	SFO	43.5	144.4	40.7	
LUFA 2.3	Sandy loam	6.7	FOMC	9.6	99.2	23.0	
LUFA 5M	Sandy loam	7.3	DFOP	6.3	73.7	15.9	
Bondhay	Silt loam	6.8 (KCl)	SFO	16.5	74.7	22.5*	Waring 1993b; (C022365)
Sandy loam	Sandy loam	5.75(KCl)	HS	16.2	53.9	16.2	Waring 1993a, (R014527)
Emerainville 2ppm	Loam	7.3 (H_2O)	DFOP	38.4	198.5	56.9*	Gouot et al., (C022418)
Emerainville 5ppm			DFOP	39.3	158.2	47.7*	Gouot et al., (C022418)

* FOMC DT_{90}/3.32

CA 7.1.2.1.2 Aerobic degradation of metabolites, breakdown and reaction products

Studies presented in the original Annex II Dossier (1995):
Studies with the metabolites of iprodione which were still considered as relevant are summarized in this supplementary dossier to provide a complete view on the fate of iprodione in the environment.

Report: Cooper, J.L.D. et al. (1998): [^{14}C]-RP 030228: Rate of degradation in three soil types under aerobic conditions.
Report Rhône-Poulenc Agriculture 12113 of June 11, 1998.
C022385.
GLP: Yes

RP 30228 is the major breakdown product of iprodione in soil. This study was designed to produce data on the degradation rate of RP 30228 under aerobic conditions in three contrasting soil types, in the absence of light. The study satisfies the requirements set out in Part G, Paragraph G 1.1 of the Dutch Pesticide Guidelines (1991).
The study was conducted in three soils, a sandy loam, a clay loam and a sand, all obtained from within the UK at a temperature of 20 ± 1°C and a moisture content equivalent to pF 2.5.

Table 7.1.2.1.2-1: Summary of soil characteristics

Soil reference	97/02	97/05	97/14
Adress of source	Aldhams Farm Manningtree, Essex, UK	Boarded Barns Farm, Ongar, Essex, UK	Fengate Farm, Weeting, Brandon, Suffolk, UK
Pesticide application history	None for 5 years	None for 7 years	None
Particle size distribution ADAS/(USDA) % sand % silt % clay	 52.33/(60.66) 39.51/(31.18) 8.16/(8.16)	 25.15/(33.67) 50.69/(42.17) 24.16/(24.16)	 89.38/(90.19) 5.49/(4.68) 5.13/(5.13)
Textural classification ADAS/(USDA)	Sandy loam/(sandy loam)	Clay loam/(loam)	Sand/(sand)
% Organic carbon	1.2	1.7	2.4
% Organic matter	2.1	2.9	4.1
pH (water) 1:5	6.5	7.2	6.9
pH (KCl, 1M) 1:5	5.3	7.0	6.3
pH ($CaCl_2$, 0.01M) 1:5	5.5	7.0	6.2
Cation exchange capacity [Meq/100g] Ca Mg Na K Mn	5.3 4.2 0.4 0.1 0.6 < 0.05	23.1 20.0 0.8 0.1 2.2 < 0.05	13.2 11.1 1.2 0.2 0.7 < 0.05
% Moisture content at pF value of 2.5	19.03	26.51	9.92
Biomass [µg C/g soil] (at beginning of study)	149	245	307
Biomass [µg C/g soil] (at end of study)	618	n/d	n/d

Results: The metabolism of [^{14}C]-RP 30228, applied at a rate of 1 kg/ha, was followed in all three soils for 100 days. In all experiments the RP 30228 degradation was limited: after 100 days the amount of RP 30228 present was > 90% of applied dose; [^{14}C]-labelled carbon dioxide and organic volatiles were all 0.2% or less. All three soils were considered to be microbially viable at the beginning of the study, therefore the breakdown of RP 30228 by soil microflora and fauna could have taken place.

Conclusion: Under laboratory conditions, in the dark at 20°C and a moisture content of 9.92 at pF 2.5, RP 30228 has limited degradation in the three contrasting soil types used. Given the results obtained it proved impossible to calculate meaningful DT_{50} and DT_{90} values. This study was conducted under laboratory conditions in the absence of light; under field conditions other mechanisms may be responsible for the degradation of RP 30228.

For RP 30228, normalised $DegT_{50}$ values between 14.6 (pH 5.5) were derived during kinetic evaluation [*Budde (2013b) – 2013/1311391*, CA 7.1.2.1.1/2] of the aerobic soil degradation study performed according to latest guidelines using latest methods [*FOCUS (2006)]*.

The degradation of RP 30228 was observed in hydrolysis studies (see chapter CA 7.2.1.1 for details).

Considering the above observations, the degradation of RP 30228 could have been expected in the study by Cooper et al. [*Cooper et al. (1998) – C022385*]. Thus, following the weight of evidence approach, the results by Cooper are considered as not appropriate for the calculation of trigger of modelling endpoints. Thus, kinetic evaluation according to FOCUS [*FOCUS (2006)*] was not performed.

Report: Quarmby D. L: Iprodione metabolite: 14C-3,5-Dichloroaniline aerobic soil metabolism
B002966
Guidelines: EPA 162-1
GLP: yes

Executive Summary

The metabolism of ^{14}C-3,5-dichloroaniline in two aerobic soils was studied over a nine month period.

Soil samples were treated with ^{14}C-3,5-dichloroaniline equivalent to 3.5 lb. a.s./acre or 3926 g a.s./ha. The samples were incubated in an environmental chamber in the dark at 25 ± 1°C and 75 ± 5% of 1/3 bar water holding capacity. A flow-through system with humidified air was used. The effluent air passed through ethylene glycol and 2-ethoxyethanol:ethanolamine (2:1 v/v) to trap organic volatiles and liberated CO_2, respectively. Duplicate samples were analysed at intervals of 0, 1, 3, 7, and 14 days and at 1, 2, 3, 6, and 9 months after application.

The average material balance ranged from 90% to 104% of the applied dose in the silt loam and 82% to 99% of the applied dose in the sandy loam soil. Volatile radioactivity accounted for less than 5% of applied activity present in any CO_2 trap and less than 0.01% present in the trap for organic volatiles.3,5-dichloroaniline was identified by HPLC and confirmed by LC/MS/MS by comparison with authentic reference standard. No degradation products were observed in either of the two soils. The parent compound was mainly degraded by converting to bound (non-extractable) residues. The non-extractable residues (NER) in the silt loam were mainly found in the soil humin fraction (50-60%), followed by the humic acid fraction (23-31%) and fulvic acid fraction (9-16%). In the sandy loam, the non-extractable residues increased with time in the humic acid fraction from 39% to 56%. This corresponded with a decrease in the soil humin fraction from 40% to 24%. The fulvic acid fraction remained stable throughout the study with 20-23% of the non-extractable residues.

The 3,5-dichloroaniline degraded in silt loam and sandy loam soils following apparent first order kinetics. Calculated half-lives were 6 days and 17 days in the silt loam soil and the sandy loam soil, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Internal code:
CAS Number.: 626-43-7
Chemical name (IUPAC): 3,5-dichloroaniline
Molecular weight: 162.02 g/mol (unlabelled)
Position of radiolabel: phenyl - U-^{14}C
Specific radioactivity: 28 mCi/mmol (~381,584 dpm/µg)
Chemical purity: 99.2% (unlabelled)
Radiochemical purity: >99%

2. Soils

Two soils were obtained freshly from Madera, California and Columbia, Missouri. The soils were sieved through a 2-mm sieve to remove extraneous debris. Soil characteristics are summarised in Table 7.1.2.1.2-2.

Table 7.1.2.1.2-2: Physicochemical characteristics of test soils

Designation	ABC Soil, #221	ABC Soil, #222
Origin	Columbia, Missouri	Madera, California
USDA particle size distribution [%] Sand > 0.002 mm Silt 0.002 – 0.050 mm Clay < 0.002 mm Textural class	 11.4 65.0 23.6 silt loam	 56.4 34.0 9.6 sandy loam
Total organic matter [%]	4.49	0.59
Soil microbial biomass (initial) [mg C/100 g soil]	35.8	5.14
Soil microbial biomass (final) [mg C/100 g soil]	18.3	6.50
CEC [meq/100g]	16.52	3.90
pH (H_2O)	6.9	7.3
WHC [%]	27.45* 12.25**	7.00* 2.03**
Bulk density [g/cm³]	1.14	1.57

* determined at 0.33 bar
** determined at 15 bar

B. STUDY DESIGN

1. Experimental conditions

The dose solution of ^{14}C-3,5-dichloroaniline was prepared in a quantity sufficient to treat the soil containers at a rate of 3.5 ppm. Portions of 100 g soil (dry weight basis) were then filled into straight-sided flasks. For each type of soil, two replicate samples for each sampling time were prepared. In addition, 4 samples for untreated controls and 8 contingency samples were prepared. Two additional samples were prepared for the end-of-study biomass determination. The moisture of soil was brought up to 75±5% of 1/3 bar water holding capacity.

The untreated control and the treated containers were connected to an aerobic soil incubation apparatus. The two soil samples for each time point were connected in parallel to the hydrated air supply. The containers were connected to a series of two volatile traps. The first trap contained ethylene glycol the second trap 2-ethoxyethanol:ethanolamine (2:1 v/v) for trapping organic volatiles and CO_2, respectively. The prepared test systems were placed in an environmental chamber at 25±1°C in the dark and allowed to acclimatise for about one week prior to dosing.

Each of the test containers received 750 µI of the ^{14}C-3,5-dichloroaniline treatment solution containing 350 µg a.s. equivalent to the maximum anticipated field concentration of , approximately 3.5 Ib. a.s./acre or 3926 g a.s./ha.

The treated and untreated control containers were incubated in an environmental chamber in the dark at 25±1°C. Soil moisture was maintained at 75±5% of 1/3 bar water holding capacity.

2. Sampling

Treated soils were sampled at 0, 1, 3, 7, and 14 days; and at 1, 2, 3, 6, and 9 months. Air flow to the sample containers was discontinued prior to removal of the soil samples from the environmental chamber. Two untreated samples were analysed at the beginning of the study and one untreated sample was analysed at the end of the study as controls.

3. Description of analytical procedures

All soils were initially extracted on the same day that they were sampled. Subsequent extractions and analyses were completed within three months of sampling.

Samples were extracted three times with an acetonitrile/water (80:20 (v/v)) solution. The liquid phase was separated from the solid phase by centrifugation, and the three extracts were combined and the total volume of the liquid extract was noted. Three replicated aliquots were taken for LSC analysis.

The soil residues were additionally extracted with methanol in a Soxhlet apparatus and placed on a heating mantle for 6 hours. The volume of the extracts was measured and aliquots were removed for liquid scintillation analysis (LSA). Since all extracts contained less than 10% of the applied radioactivity, they were not further analysed by HPLC.

Radioactive residues remaining in the soil samples after extractions were determined by combustion and subsequent analysis by LSA. The trapping solutions were directly counted by LSA to account for volatile ^{14}C-residues.

When less than 90% of the applied radioactivity was recovered from the soil and traps combined, the Tygon tubing used to connect the traps was analysed. The Tygon tubing from each test system was cut into 0.5-1.0 inch segments and extracted with mL of warm methanol. The methanol was decanted and aliquots removed for LSC. Some test systems demonstrated relatively low material balances, so an attempt was made to try to extract additional activity from the tubing. For these systems, samples were further extracted with mL acetone and measured by LSA. Each of the samples was next extracted with HPLC grade water and determined with LSC. Finally, acidified methanol (pH 4, adjusted with phosphoric acid) was added to each tubing sample. The samples sat at room temperature for 30 minutes, and then shaken for 24 hours before the solvent was decanted and counted by LSC.

Bound residues remaining in the soil samples after extraction and combustion were characterised into fulvic acid, humic acid, and soil humin. Aliquots of the extracted air-dried soils were treated with 0.1 N NaOH and the solid phase was separated from the supernatant by centrifugation. The remaining soil pellet was treated with deionised water and the phases were separated as above. The remaining soil pellet was air dried and combusted to determine the amount of soil humin.

The water and NaOH supernatants were combined and the pH was adjusted to pH 2 with 2N HCI. The samples were then separated by centrifugation and aliquots from the supernatant were removed for LSA to determine the amount of fulvic acid. Humic acid in the remaining pellet was determined by combustion.

All radiometric results were expressed as parent equivalents. Initial extracts from all time points were analysed by HPLC. The parent compound identified by HPLC was confirmed by mass spectrometry (LC/MS/MS) analysis.
The limit of detection (LOD) for analysis of ^{14}C-3,5-dichloroaniline equivalents in combusted soil residues was based on two times the value of background dpm. The LOD was calculated to be <0.01 ppm. The LOD and LOQ for soil extracts were calculated from the control extracts. The detection limit for HPLC was calculated to be 0.002 ppm.

4. Calculation of the degradation rate

The parent concentration at various time intervals was subjected to correlation and regression analysis. Rate constant and half-life ($t_{1/2}$) were calculated by using a linear regression, assuming pseudo first-order reaction kinetics. The disappearance time DT_{50} (time needed for 50% of degradation) was calculated using the following equation: DT_{50} = Ln2/k. The time for 90% disappearance (DT_{90}) was calculated by the following equation: DT_{90} = Ln10/k.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The material balance of the radiocarbon recovered in the volatile traps, Tygon tubing, extractables, and bound ^{14}C-residues was expressed as per cent of applied dose. The average total recovery of ^{14}C-residues ranged from 90% to 104% of the applied dose in the silt loam and 82% to 99% of the applied dose in the sandy loam soil. The detailed results on material balance in the silt loam and sandy loam soils are presented in Table 7.1.2.1.2-3 and Table 7.1.2.1.2-4, respectively.

B. EXTRACTABLE AND BOUND RESIDUES

The distribution of radioactivity in extractable residues (ERR), non-extractable residues (NER), and volatiles for the silt loam and sandy loam soils is shown in Table 7.1.2.1.2-3 and Table 7.1.2.1.2-4, respectively. At day 0, 88% of applied radioactivity was recovered with the initial extract in the silt loam and 95% was recovered in the sandy loam. By the 9 months sampling time, only 8-10 % was extracted from either soil. About 1 to 7 % of applied activity was recovered from the soil using methanol soxhlet extraction. In addition, the tubing which connected the traps was extracted and found to contain between 0 to 7 % of applied activity.

Table 7.1.2.1.2-3: Material balance of radioactivity from aerobic soil metabolism for silt loam soil [% TAR]

Sampling time [day or month]	ERR		Volatiles			NER	Total Recovery
	ACN/H_2O	Methanol Soxhlet	Trap 1	Trap 2	Tubing	Bound residues	
0 days	87.66	4.01	-	-	N/A	5.96	
0 days	88.57	3.70	-	-	N/A	6.35	
	88.12	**3.86**	**ND**	**ND**	**N/A**	**6.16**	**98.14**
1 day	66.87	7.17	-	-	-	21.77	
1 day	65.89	6.34	-	-	-	20.68	
	66.38	**6.76**	**0.00**	**0.03**	**0.09**	**21.22**	**94.48**
3 days	49.23	7.06	-	-	-	34.13	
3 days	48.69	8.36	-	-	-	36.25	
	48.96	**7.71**	**0.00**	**0.10**	**0.13**	**35.19**	**92.09**
7 days	37.18	6.48	-	-	-	46.09	
7 days	38.08	6.99	-	-	-	44.08	
	37.63	**6.73**	**0.00**	**0.00**	**0.43**	**45.08**	**89.87**
14 days	16.40	5.41	-	-	-	62.11	
14 days	17.39	5.96	-	-	-	62.77	
	16.90	**5.68**	**0.00**	**1.20**	**5.39**	**62.44**	**91.61**
1 month	13.06	4.51	-	-	-	73.23	
1 month	13.59	4.31	-	-	-	70.97	
	13.33	**4.41**	**0.01**	**1.42**	**2.76**	**72.10**	**94.03**
2 months	10.25	3.10	-	-	-	82.60	
2 months	10.55	3.22	-	-	-	79.44	
	10.40	**3.16**	**0.00**	**2.32**	**6.67**	**81.02**	**103.57**
3 months	9.88	2.73	-	-	-	75.21	
3 months	9.74	2.40	-	-	-	77.17	
	9.81	**2.57**	**0.01**	**3.20**	**4.12**	**76.19**	**95.90**
6 months	8.67	1.79	-	-	-	76.06	
6 months	8.50	1.41	-	-	-	76.13	
	8.59	**1.60**	**0.01**	**1.90**	**5.05**	**76.10**	**93.25**
9 months	7.72	2.17	-	-	-	75.86	
9 months	7.87	1.75	-	-	-	75.75	
	7.80	**1.96**	**0.01**	**1.58**	**3.11**	**76.81**	**90.27**

ERR = extractable residues
NER = non-extractable residues
Trap 1 = ethylene glycol
Trap 2 = 2-ethoxyethanol:ethanolamine
Traps were combined for each sample set.
Bold numbers = mean values

Table 7.1.2.1.2-4: Material balance of radioactivity from aerobic soil metabolism for sandy loam soil [% TAR]

Sampling time [day or month]	ERR		Volatiles			NER	Total Recovery
	ACN/H_2O	Methanol Soxhlet	Trap 1	Trap 2	Tubing	Bound residues	
0 days	95.13	2.02	-	-	-	2.16	
0 days	95.54	1.53	-	-	-	2.21	
	95.33	**1.78**	**ND**	**ND**	**N/A**	**2.19**	**99.30**
1 day	82.48	3.12	-	-	-	8.86	
1 day	81.49	3.56	-	-	-	8.52	
	81.98	**3.34**	**0.01**	**0.02**	**2.39**	**8.69**	**96.43**
3 days	67.28	4.73	-	-	-	14.12	
3 days	67.63	4.30	-	-	-	15.26	
	67.45	**4.51**	**0.00**	**0.13**	**0.74**	**14.69**	**87.52**
7 days	54.45	3.30	-	-	-	25.47	
7 days	56.67	3.20	-	-	-	25.01	
	55.56	**3.25**	**0.00**	**0.32**	**4.55**	**25.24**	**88.92**
14 days	40.00	4.23	-	-	-	36.53	
14 days	43.65	3.71	-	-	-	41.00	
	41.83	**3.97**	**ND**	**0.73**	**2.13**	**38.77**	**87.43**
1 month	24.68	3.46	-	-	-	59.20	
1 month	27.00	3.36	-	-	-	58.97	
	25.84	**3.41**	**0.00**	**1.52**	**3.43**	**59.09**	**93.29**
2 months	17.11	1.95	-	-	-	65.89	
2 months	16.86	3.63	-	-	-	66.38	
	16.99	**2.79**	**0.00**	**2.41**	**3.85**	**66.14**	**92.18**
3 months	12.50	1.54	-	-	-	60.01	
3 months	12.63	1.05	-	-	-	60.04	
	12.57	**1.30**	**0.01**	**2.90**	**7.38**	**60.03**	**84.19**
6 months	11.05	1.19	-	-	-	64.03	
6 months	10.98	1.20	-	-	-	62.02	
	11.02	**1.20**	**0.01**	**4.87**	**4.90**	**63.03**	**85.03**
9 months	9.57	2.22	-	-	-	64.63	
9 months	9.52	1.22	-	-	-	65.19	
	9.55	**1.72**	**0.02**	**3.36**	**2.71**	**64.91**	**82.27**

ERR = extractable residues
NER = non-extractable residues
Trap 1 = ethylene glycol
Trap 2 = 2-ethoxyethanol:ethanolamine
Traps were combined for each sample set.
Bold numbers = mean values

The remaining non-extractable soil residues were characterised into fulvic acid, humic acid, and soil humin. In the silt loam, most of the nonextractable residues were found in the soil humin fraction (50-60%), followed by the humic acid fraction (23-31%) and fulvic acid fraction (9-16%). In the sandy loam, the nonextractable residues increased with time in the humic acid fraction from 39% to 56%. This corresponded with a decrease in the soil humin fraction from 40% to 24%. The fulvic acid fraction remained stable throughout the study with 20 to 23% of the non-extractable residues. A summary of organic matter characterisation for 3,5-dichloroaniline in the silt loam and the sandy loam soils is given in Table 7.1.2.1.2-5 and Table 7.1.2.1.2-6, respectively.

Table 7.1.2.1.2-5: Organic matter characterisation summary for 3,5-dichloroaniline aerobic soil metabolism in silt loam soil

Sampling time [day or month]	Fulvic acid		Humic acid		Soil humin		Total per cent of applied
	% of applied	% of total residue	% of applied	% of total residue	% of applied	% of total residue	
0 days	0.51	9.2	1.33	23.9	3.73	67.0	5.57
1 day	1.89	10.4	4.21	23.2	12.07	66.4	18.17
3 days	3.28	10.6	9.71	31.3	18.06	58.2	31.05
7 days	4.35	11.3	10.83	28.2	23.28	60.5	38.46
14 days	6.55	12.1	14.05	26.2	33.03	61.6	53.63
1 month	6.49	11.3	17.02	29.7	33.71	59.0	57.22
2 months	7.79	14.7	17.57	33.1	27.77	52.3	53.13
3 months	7.47	12.2	19.01	31.1	34.59	56.7	61.07
6 months	7.93	15.6	14.78	29.0	28.25	55.4	50.96
9 months	8.02	15.8	16.00	31.5	26.73	52.7	50.75

values given are the average of the two silt loam samples analysed at each sampling time.

Table 7.1.2.1.2-6: Organic matter characterisation summary for 3,5-dichloroaniline aerobic soil metabolism in sandy loam soil

Sampling time [day or month]	Fulvic acid		Humic acid		Soil humin		Total per cent of applied
	% of applied	% of total residue	% of applied	% of total residue	% of applied	% of total residue	
0 days	0.37	19.6	0.79	41.8	0.73	38.6	1.89
1 day	1.63	21.0	3.00	38.7	3.13	40.3	7.76
3 days	3.03	20.8	6.23	42.7	5.33	36.5	14.59
7 days	4.70	20.5	9.46	41.3	8.73	38.1	22.89
14 days	6.69	21.5	14.01	45.0	10.42	33.5	31.12
1 month	9.43	20.6	19.90	43.4	16.53	36.0	45.86
2 months	13.03	23.2	27.72	49.5	15.28	27.3	56.03
3 months	11.91	20.7	30.79	53.4	14.93	25.9	57.63
6 months	10.56	20.0	29.69	56.1	12.64	23.9	52.89
9 months	11.54	21.7	26.38	49.5	15.33	28.8	53.25

values given are the average of the two silt loam samples analysed at each sampling time.

C. VOLATILISATION

Volatile organic radioactivity remained at less than 10% throughout the test period. The first trap (ethylene glycol) accounted for 0.01% or less of the applied radioactivity at all time points. The second trap (2-ethoxyethanol:ethanolamine) accounted for up to 3% of applied activity in the silt loam soil and up to 5% of applied activity in the sandy loam soil.

D. TRANSFORMATION OF PARENT COMPOUND

The decline of ^{14}C-3,5-dichloroaniline in silt and sandy loam soils is presented in Table 7.1.2.1.2-7. The 3,5-dichloroaniline degraded quickly in the aerobic soil environment.

The rate of 3,5-dichloroaniline degradation was calculated using the data obtained from 0 to 14 days for the silt loam soil, at which time only 17% of the applied radioactivity was extractable. The rate of 3,5-dichloroaniline degradation was calculated using the data obtained from 0 to 1 month for the sandy loam soil where only 26% was extractable at this time.

Table 7.1.2.1.2-7: Pattern of decline of 3,5-dichloroaniline in silt loam and sandy loam soils under aerobic conditions

Sampling time [day or month]	Silt loam soil	Sandy loam soil
	[%TAR]	[%TAR]
0 days	88.12	95.33
1 day	6638	81.98
3 days	48.96	67.45
7 days	37.63	55.56
14 days	16.90	41.83
1 month	13.33	25.84
2 months	10.40	16.99
3 months	9.81	12.57
6 months	8.59	11.02
9 months	7.80	9.55

In the silt loam soil, 3,5-dichloroaniline had a calculated half-life of 6 days and a DT_{90} of 21 days. In the sandy loam soil, the half-life of 3,5-dichloroaniline was 17 days, with a DT_{90} of 56 days.

III. CONCLUSION

Summing up the results of all investigations in this study it can be concluded that 3,5-dichloroaniline is aerobically degradable in a silt loam soil with a half-life of 6 days and in a sandy loam soil and with a half-life of 17 days. The DT_{90} values for the two soils are 21 and 56 days, respectively.

Volatile radioactivity accounted for less than 5 % of applied activity present in any CO_2 trap and less than 0.01% present in the trap for organic volatiles.

Only 3,5-dichloroaniline was present in the soil extracts. No other degradation products were observed in either of the two soils. The test item was mainly degraded to non-extractable residues.
In the silt loam, most of the non-extractable residues were found in the soil humin fraction (50-60%), followed by the humic acid (23-31%) and fulvic acid fraction (9-16%). In the sandy loam, the non-extractable residues increased with time in the humic acid fraction from 39% to 56%. This corresponded with a decrease in the soil humin fraction from 40% to 24%. The fulvic acid fraction remained stable throughout the study with 20-23% of the non-extractable residues.

The kinetic analysis of this study was repeated by Szegedi [*Szegedi (2014) – 2013/1311394, CA 7.1.2.1.1/3*] considering latest guidance documents. The determined half-life (trigger $DegT_{50}$) is 2.7 d for the Columbia silt loam soil and 8.6 d for the Modera sandy loam soil. Details are given in chapter 7.1.2, CA 7.1.2.1.1./2.

Report: Gouot, J.M. et al. (1981): The behaviour of 3,5-dichloroaniline (RP 32596) in a sandy loam soil of Dutch origin.
Report Rhône-Poulenc Agriculture P93/073 of July 15 1993.
C022371
GLP: Yes

The study was carried out with sandy loam soil, rich in organic matter at a dose of 10 ppm for 5 months.

Results: The 3,5-dichloroaniline rapidly became unextractable from the soil and the apparent half-life was estimated to be about 2 weeks. The total extractable radioactivity decreased in parallel. There were few volatile compounds, these principally being detected in the alkaline traps for the CO_2 and representing a mean level of 0.3% of the total radioactivity per month. The losses of radioactivity observed by T.L.C. were attributed to losses of 3,5-dichloroaniline, after comparison with the results obtained by G.L.C. and H.P.L.C.

Very polar compounds were observed on the T.L.C. plates. The total quantity reached a maximum of 10% of the initial radioactivity but this figure should be corrected to allow for the presence of artefacts on the plates. A compound derived from 3,5-dichloroaniline, present in small quantities (1 to 3% of the initial radioactivity) was identified as N-(3,5-dichlorophenyl)formamide.

The unextractable radioactivity increased rapidly during the first 3 months to reach a level of 65% of the initial radioactivity after 5 months.

The kinetic analysis of this study was repeated by Szegedi [*Szegedi (2014) – 2013/1311394, CA 7.1.2.1.1/3*] considering latest guidance documents. The determined half-life (trigger $DegT_{50}$) is 8.6 d. Details are given in chapter 7.1.2, CA 7.1.2.1.1./2.

Not yet peer-reviewed studies

Report:	CA 7.1.2.1.2/1 Class T., 2013a Aerobic soil degradation of Reg.No. 5079618 (metabolite of Iprodione: RP 36221) in three soils (OECD Guideline 307) 2013/1211061
Guidelines:	OECD 307 (2002), BBA IV 4-1, EPA 835.4100, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The objective of this study was to determine the rate of aerobic degradation of Reg. No. 5079618 (metabolite of iprodione, RP 36221) in three soils.

Three soils were used for this study: Loamy sand (Li10), loamy sand (LUFA 5M) and loamy sand (LUFA 2.3) (soil classes according to DIN). The test substance was applied to a bulk soil based on soil dry weight to achieve a nominal application rate of 0.5 mg/kg, which corresponds to a theoretical field application rate of 200 g/ha. The treated soils were incubated at 40% maximum water holding capacity (MWHC) at 20°C in the dark up to 120 days. After given incubation periods, replicate soil incubations were collected and soil was extracted for subsequent LC/MS/MS determination of the analyte. The analytical method was validated for soil extraction and determination of Reg. No. 5079618 and achieves a limit of quantitation (LOQ) of 0.005 mg/kg for Reg. No. 5079618.
The DT_{50} values for the degradation of Reg. No. 5079618 were calculated. The trigger endpoints derived from FOMC modelling result for soils LUFA 2.3 and Li 10 in DT_{50} of > 1000 days. The trigger endpoint for Reg. No. 5079618 derived from SFO modelling results for LUFA 5M in DT_{50} of 715 days.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Reg. No.: 5079618
CAS Number.: 63637-88-7
Chemical name (IUPAC): 1-(3,5-dichlorophenyl)-5-isopropyl biuret
Molecular weight: 290.1 g/mol
Chemical purity: 92.2%

2. Soils

Soils used in this study were loamy sand (Li10), loamy sand (LUFA 5M) and loamy sand (LUFA 2.3) (soil classes according to DIN). Soils were first kept under aerobic conditions at about 5°C for about 21 days, then soils were adjusted to 40 % of their maximum water holding capacities and the soils were acclimatised for 14 days at room temperature in the dark. The soil characteristics are summarised in Table 7.1.2.1.2-8.

Table 7.1.2.1.2-8: Soil Characteristics

Parameter	Li 10	LUFA 5M	LUFA 2.3
Soil type	Loamy sand (DIN)	Loamy sand (DIN)	Loamy sand (DIN)
Particle size distribution [%]			
Sand (63 - 2000 µm)	82.8	54.1	59.3
Silt (2 - 63 µm)	11.7	31.7	30.4
Clay (< 2 µm)	5.5	14.2	10.3
Soil type	Loamy sand (USDA)	Sandy loam (USDA)	Sandy loam (USDA)
Particle size distribution [%]			
Sand (50 - 2000 µm)	83.5	58.4	63.8
Silt (2 - 50 µm)	11.0	27.4	25.9
Clay (< 2 µm)	5.5	14.2	10.3
Organic carbon [%]	0.81	2.18	1.09
Microbial biomass [mg C/kg dry soil]	33.6	31.1	32.8
CEC [$cmol^+/kg$]	5.0		7.7
pH ($CaCl_2/H_2O$)	6.3/7.1	7.4/8.0	7.0/7.7
Max. water holding capacity [g/100g soil]	23.1	26.2	25.4

B. STUDY DESIGN

1. Experimental conditions

The test substance was applied as small doses (100 µL) taken from a solution of 500 µg/mL in acetone. In total, 2.0 mL were applied to 2 kg of soil bulk mass based on soil dry weight. The soil was homogenised for five minutes using a hand-hold mixer after each dosing. After homogenisation, soils water content was determined gravimetrically and re-adjusted.
The application rate (based on dry soil weight) of Reg. No. 5079618 dosed to soil was 0.5 mg/kg. Assuming a soil depth of 2.5 cm and a density of 1.5 g/cm^3 this rate correspond to a theoretical field application rate of about 200 g/ha.
The 50-g of dry soil mass equivalents in the incubation 1000-mL vessels were loosely covered with a plug of paper tissue to prevent excessive loss of water and to allow exchange of air and then incubated at 20±1 °C in a thermostatic cabinet in the dark.

2. Sampling

Duplicate soil samples were taken at 0, 2, 7, 14, 30, 60, 90, and 120 days after treatment (DAT).

3. Description of analytical procedures

An analytical LC-MS/MS-based method previously developed and validated at PTRL Europe in a related study was adapted for soil extraction and determination of Reg. No. 5079618.

The soil samples were extracted once with 20 mL of acetonitrile/water (1/1 v/v, 0.1% formic acid) and twice with 20 mL of acetonitrile containing 0.1% formic acid. . The solvent extracts were pooled by decanting the supernatant through a glass funnel fitted with silanised glass wool, followed by concentration and analysis by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS). An aliquot of the final extract was diluted by a factor DF of 10 and injected for LC-MS/MS analysis.

4. Calculation of the degradation rate

The calculation of the DT_{50} and DT_{90} values was performed following the recommendations of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. Optimisation of model parameters, including estimation of parameter standard errors, was performed using the software package KinGUI version 1.1. Triggers were taken from the best fit kinetics, while modelling endpoints derived from the selected kinetic model based on visual and statistical acceptance.

II. RESULTS AND DISCUSSION

A. LC-MS/MS Results

Table 7.1.2.1.2-9 to Table 7.1.2.1.2-11 give LC-MS/MS results for soil method concurrent validation and for soil degradation, including the residues of the analyte remaining and extracted after given incubation periods. These tables also show the remaining analyte as percentage of the initially applied residue (%AR: nominally about 0.5 mg/kg of Reg. No. 5079618) at the beginning of the incubation.

B. Kinetics Analyses

The residues observed for the analyte (expressed as µg/kg) in the incubated soil samples were fitted by using the software package KinGUI version 1.1.

The DT_{50} values derived from kinetic evaluation according to FOCUS [*FOCUS (2006)*] were 715 days in soil LUFA 5M (SFO) and >1000 days in the other two soils (FOMC). The SFO-DT_{50} of 715 days (LUFA 5M) is appropriate to be used as modelling endpoint, whole for soils Li10 and LUFA 2.3 no significant parameters could be obtained.

Table 7.1.2.1.2-9: Degradation in soil Lufa 2.3

Time [days]	Residues [mg/kg]	Residues [%AR]
0	0.518	102
0	0.502	99
2	0.513	101
2	0.516	101
7	0.504	99
7	0.507	100
14	0.484	95
14	0.477	94
30	0.424	83
30	0.459	90
60	0.449	88
60	0.424	83
90	0.425	84
90	0.425	84
120	0.440	87
120	0.430	85

Table 7.1.2.1.2-10: Degradation in soil Lufa 5M

Time [days]	Residues [mg/kg]	Residues [%AR]
0	0.508	97
0	0.523	100
2	0.499	95
2	0.582	111
7	0.550	105
7	0.549	105
14	0.501	96
14	0.530	101
30	0.479	92
30	0.490	94
60	0.443	85
60	0.486	93
90	0.502	96
90	0.450	86
120	0.514	98
120	0.468	89

Table 7.1.2.1.2-11: Degradation in soil Li10

Time [days]	Residues [mg/kg]	Residues [%AR]
0	0.536	106
0	0.513	101
2	0.574	113
2	0.526	104
7	0.525	104
7	0.538	106
14	0.513	101
14	0.507	100
30	0.491	97
30	0.500	99
60	0.540	107
60	0.463	91
90	0.517	102
90	0.519	102
120	0.513	101
120	0.514	101

III. CONCLUSION

The objective of the study was to examine aerobic degradation of Reg. No. 5079618 (metabolite of iprodione) in 3 different soils. The residues observed for the analyte (expressed as µg/kg) in the incubated soil samples were fitted using the software package KinGUI version 1.1. The trigger endpoints for Reg. No. 5079618 derived from FOMC modelling result for soils LUFA 2.3 and Li10 in DT_{50} of > 1000 days. The trigger endpoint for Reg. No. 5079618 derived from SFO modelling results for LUFA 5M in a DT_{50} of 715 days.

Report:	CA 7.1.2.1.2/2 Class T., 2013b Aerobic soil degradation of Reg.No. 207099 (metabolite of Iprodione: RP 25040) in three soils (OECD Guideline 307) 2013/1078064
Guidelines:	OECD 307 (2002), BBA 4-1.1.3, EPA 835.4100, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The objective of this study was to determine the rate of aerobic degradation and degradation half-lives for iprodione metabolite Reg. No. 207099 (former RP 25040) in three soils.

Three field-fresh soils were investigated: one loamy sand (soil Li10) and two sandy loams (soils LUFA 2.3 and LUFA 5M). Soil moistures were adjusted to 40% of the maximum water holding capacity. The test substance was applied at a nominal application rate of 0.5 mg/kg, which corresponds to a theoretical field application rate of 200 g/ha.

Test systems containing 50 g of treated soils (based on dry soil equivalents) were incubated at 20 °C for various intervals up to 21 days in the dark. Collected duplicate samples were extracted and analysed in replicates for the test substance using LC-MS/MS. The analytical method was validated for soil extraction determination of iprodione metabolite Reg. No. 207099. The mean recoveries found for the 0.005 mg/kg and 0.50 mg/kg fortifications for all three soils were 107% and 105% (n=9).

Iprodione metabolite Reg. No. 207099 was degraded in all the three soils tested. The amount of the test substance decreased to a value less than 10% of the initial applied radioactivity in soil LUFA 2.3, and less than 5% of the initial applied for both soil LUFA 5M and Li10 at the end of incubation periods.

The kinetic analysis of measured residues showed that degradation of iprodione metabolite Reg. No. 207099 was best described using FOMC kinetics. The modelling half-life times (DT_{50}) calculated from FOMC DT_{90} were 0 4.1, 0.75 and 1.7 days for Li 10, LUFA 5M and LUFA 2.3, and Li10, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Internal code: Reg.No. 207099 (former RP 25040)

CAS Number.: 27387-87-7
Chemical name (IUPAC): 3-(3,5-dichlorophenyl)-2,4-dioxoimidazolidine
Molar mass: 245.1 g/mol
Chemical purity: 99.6% ± 1.0%

2. Soils

Three different field-fresh soils were used in this study. Samples were kept under aerobic conditions at about 5 °C for about 21 days. Soils moisture content was adjusted to 40% of their maximum water holding capacity and acclimatised for 9 days at room temperature in the dark. Soil characteristics are summarised in Table 7.1.2.1.2-12.

Table 7.1.2.1.2-12: Soil Characteristics

Soil designation Origin	**Li10**	**LUFA 5M**	**LUFA 2.3**
Textural class (German scheme, DIN 4220)	Loamy sand (Sl2)	Loamy sand (Sl4)	Loamy sand (Sl3)
Soil texture [%], (German scheme)			
Sand	82.8	54.1	59.3
Silt	11.7	31.7	30.4
Clay	5.5	14.2	10.3
Textural class (USDA scheme)	Loamy sand	Sandy loam	Sandy loam
Soil texture [%], (USDA scheme)			
Sand	83.5	58.4	63.8
Silt	11.0	27.4	25.9
Clay	5.5	14.2	10.3
Microbial biomass [mg C/kg dry soil]	33.6	31.1	32.8
Organic carbon [%]	0.81	2.18	1.09
CEC [$cmol^+/kg$]	5.0	8.8	7.7
pH ($CaCl_2$)	6.3	7.4	7.0
pH (water)	7.1	8.0	7.7
Max. water holding capacity [g/100g soil]	23.1	26.2	25.4
Bulk density [g/L]	1433	1345	1324

B. STUDY DESIGN

1. Experimental conditions

The test substance was applied in small doses (100 µL) from a 500 µg/mL solution in acetone. In total, 2.0 mL were applied to 2 kg of soil bulk mass based on soil dry weight. The soil was homogenised for five minutes using a hand-hold mixer after dosing. The water content of the soils was determined gravimetrically and re-adjusted after homogenisation. The application rate corresponded to a theoretical field application rate of about 200 g/ha assuming a soil depth of 2.5 cm and a soil bulk density of 1.5 g/cm^3.

Aliquots of 50 g treated soil (dry-mass-equivalent) were filled in 1000 mL incubation flasks and covered loosely with a paper tissue to minimize water loss but to allow air exchange and incubated in the dark at 20 ± 1°C in the dark. Loss of soil water was controlled once a week by weighing and re-adjusted with distilled water. Additionally untreated and treated moist bulks of soil were kept in the therrnostated cabinet.

The microbial biomass was determined shortly before the start of incubation, after application and at the end of incubation to check that all soils were viable throughout the incubation period.

2. Sampling

Soil test systems were incubated for different intervals up to 21 days before extraction. For soil Li10 samples were taken at 0, 1, 4, 27, 51, 146, 285, 361 and 506 hours after treatment (DAT). For soil LUFA 5M and soil LUFA 2.3 sampling times were and 0, 1.5, 4.5, 27, 51, 146, 286, 309 and 507 and 0, 2.5, 5.5, 28, 52, 147, 287, 310, and 508 respectively.

3. Description of analytical procedures

Aliquots of 10 g soil sample (based on dry mass) were extracted with 20 mL of acetonitrile/water (1/1 v/v. 0.1% formic acid) by shaking for 30 minutes on a horizontal shaker. Samples were sonicated for 10 minutes followed by centrifugation. Supernatants were transferred into volumetric flasks using a glass funnel fitted with silanised glass wool. Extraction was repeated two more times with 20 mL solution of acetonitrile containing 0.1% formic acid. Extracts were combined and diluted with acetonitrile/water (1/1 v/v. 0.1% formic acid) to a final volume of 100 mL. An aliquot of the final extract was diluted by a factor of 10 and analysed by means of LC-MS/MS.
The analytical method was validated for soil extraction determination of iprodione metabolite Reg. No. 207099. The average mean recoveries found for the 0.005 mg/kg and 0.50 mg/kg fortifications for all three soils were between were 107% and 105% (n=9).

4. Calculation of the degradation rate

Modelling endpoints were calculated according to the recommendations of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*], by fitting SFO, DFOP, FOMC, and HS kinetic models using KinGUI software version 1.1 to the residues of iprodione metabolite Reg. No. 207099 measured in soil extracts (µg/kg). Half-life-times DT_{50} were calculated from the selected kinetic model based on visual and statistical criteria.

II. RESULTS AND DISCUSSION

1. Microbial biomass

The microbial biomass of the individual soils declined slightly throughout the incubation phase, but proved that the microbial population was still viable and active throughout the study.

2. Aerobic degradation in three different soils

The analysis of the soil extracts showed degradation of iprodione metabolite Reg. No. 207099 in all three soils. The average amount of the test substance after about 21 days of incubation accounted for 6.2% of the initial applied radioactivity (AR) in soil Li10, for approximately 1% AR in soil LUFA 5M and for approximately 3.5% AR in soil LUFA 2.3 (Table 7.1.2.1.2-13 to Table 7.1.2.1.2-15).

Table 7.1.2.1.2-13: Soil residues [µg/kg] and mass balances [%AR] of soil Li10 used for kinetic analysis

DAT [hours]	Residues [µg/kg]	Residues [%AR]
0	500	100
1	534	106.9
4	485	97
4	527	105.4
27	396	79.2
27	391	78.2
51	304	60.8
51	299	59.8
146	138	27.6
146	142	28.4
285	63.6	12.7
285	64.5	12.9
361	42.7	8.54
361	48.7	9.74
506	30.9	6.18
506	31.3	6.26

Table 7.1.2.1.2-14: Soil residues [µg/kg] and mass balances [%AR] of soil LUFA 5M used for kinetic analysis

DAT [hours]	Residues [µg/kg]	Residues [%AR]
0	500	100
1.5	426	85.2
4.5	319	63.8
4.5	364	72.8
27	109	21.8
27	119	23.8
51	62.2	12.4
51	53.1	10.6
146	16.8	3.36
146	16	3.20
286	7.69	1.54
286	8.91	1.78
309	7.36	1.47
309	7.75	1.55
507	5.42	1.08
507	4.42	0.88

Table 7.1.2.1.2-15: Soil residues in [µg/kg] and as [%AR] of soil LUFA 2.3 used for kinetic analysis

DAT [hours]	Residues [µg/kg]	Residues [%AR]
0	500	100
2.5	372	74.4
5.5	321	64.2
5.5	317	63.4
28	151	30.2
28	157	31.4
52	100	20
52	95.2	19
147	44.1	8.82
147	42	8.4
287	23.2	4.64
287	23.4	4.68
310	22.8	4.56
310	21.3	4.26
508	18.7	3.74
508	15.9	3.18

Evaluation of the aerobic degradation of the analyte applying FOCUS guideline recommendations resulted in degradation times DT_{50} and statistical parameters as listed in Table 7.1.2.1.2-16. The estimated DT_{50} values for the degradation of iprodione metabolite Reg. No. 207099 were obtained using FOMC kinetics and were back-calculated from FOMC DT_{90} (DT_{50} = DT_{90}/3.32). The calculated DT_{50} values were 4.1, 0.75 and 1.7 days for Li10, LUFA 5M and LUFA 2.3 soils, respectively.

Table 7.1.2.1.2-16: Chi-squared error (X^2 error), estimated parameters and statistical results of model fit using FOMC

Data set	X^2 error	Type I error rate (prob. p > t)	DT_{50}	DT_{90}	Back calculated DT_{50} = DT_{90}/3.32
	%	-	hours	hours	hours
Li 10	3.5	<0.001	67.3	329	99.0
LUFA 5M	1.8	<0.001	9.2	60.1	18.1
LUFA 2.3	4.8	<0.001	10.5	135.5	40.8

III. CONCLUSION

Aerobic degradation of iprodione metabolite Reg. No. 207099 was examined in three soils. Kinetic analysis of the aerobic degradation data showed that iprodione metabolite Reg. No. 207099 degradation in three soils was best described using a first order multi-compartment (FOMC) kinetics. The degradation half-lives ranged from 0.75 – 4.1 days.

The compound LS 720942 (RP 44247, Reg. No. 89517) was found in iprodione photolysis on soil [John et al. (1993) – *R000277*]. As this metabolite was not detected in the soil metabolism study, degradation half-lives were determined in a separate study.

Report:	CA 7.1.2.1.2/3 Wadim W., 2014a Aerobic soil degradation of Reg. No. 89517 (metabolite of Iprodione) in three soils (OECD Guideline 307) 2014/1020546
Guidelines:	OECD 307 (2002), BBA IV 4-1, EPA 835.4100, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The objective of the study was to examine the aerobic degradation of Reg. No. 89517 (LS 720942, RP 44247), a metabolite of iprodione in three soils.

Three field-fresh soils were used in the study. The soils were classified according to DIN soil classification as: silty sand (LUFA 2.3), sandy loam (LUFA 5M) and loamy sand (Li10). Soils were acclimatised with soil moistures adjusted to 40 % of their maximum water holding capacities (MWHC). Bulk soils were treated with Reg. No. 89517 at an nominal application rate of 0.25 mg/kg based on soil dray weight. Assuming a soil depth of 2.5 cm and a soil density of 1.5 g/cm^3 this corresponds to a theoretical field application rate of about 100 g/ha.

Portions of 50 g of dosed dry soil equivalents were incubated in thermostated cabinet(s) at 20 °C in the dark for various intervals up to 59 days prior to extraction. An analytical LC/MS/MS-based method was adapted and concurrently validated for soil extraction and determination of Reg. No. 89517. A a limit of quantitation (LOQ) of 0.005 mg/kg and a limit of detection (LOD) of 0.001 mg/kg were achieved for Reg. No. 89517.

Observed soil residues for Reg. No. 89517 expressed in µg/kg were fitted using the software package KinGUI version 2.2. Single First Order kinetics (SFO) was the best-fit model for all three soils with an acceptable description of the data statistically as well as visually. The resulting trigger endpoints resulted in DT_{50} of 8.03 days, 4.22 days, and 11.12 days for the soils LUFA 2.3, LUFA 5M, and Li10, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Internal code: Reg. No. 89517

CAS Number.: 13142-57-9
Chemical name (IUPAC): (3,5-dichlorophenyl)urea
Molar mass: 205.0 g/mol (unlabelled)
Molecular formula: $C_7H_6Cl_2N_2O$
Chemical purity: 99.9 ± 1.0%

A stock solution of Reg. No. 89517 was prepared by accurately weighing and dissolving the analyte in acetone to obtain a concentration of 0.50 mg/mL. This stock solution was used to fortify soil incubation samples.

2. Soils

Three different fresh field soils were used in the aerobic degradation study. The soils were classified according to DIN classification as silty sand (LUFA 2.3), loamy sand (LUFA 5M) and loamy sand (Li10). Soils were first kept under aerobic conditions at room temperature for about 12 days, then water was adjusted to 40 % of maximum water holding capacity (MWHC) and then they were acclimatised for 5 days at 20°C in the dark with soil moistures adjusted to 40 % of their MWHC. The soil characteristics are summarised in Table 7.1.2.1.2-17.

Table 7.1.2.1.2-17: Soil Characteristics

Parameter	Lufa 2.3	Lufa 5M	Li10
Soil type	Sandy loam (USDA)	Sandy loam (USDA)	Loamy sand (USDA)
Particle size distribution [%]			
Sand (50 - 2000 µm)	64.5	60.5	84.0
Silt (2 - 50 µm)	28.6	28.5	11.0
Clay (< 2 µm)	6.9	10.9	5.0
Soil type	Silty sand (Su3) (DIN 4220)	Loamy sand (SI3) (DIN 4220)	Loamy sand (SI2) (DIN 4220)
Particle size distribution [%]			
Sand (63 - 2000 µm)	60.9	55.5	81.8
Silt (2 - 63 µm)	32.1	33.6	13.3
Clay (< 2 µm)	6.9	10.9	5.0
Bulk density [g/L]	1378	1218	1377
Dry matter [%]	91.6	92.4	92.7
Total organic carbon [%]	0.71	2.01	0.93
Microbial biomass [mg C/100 g dry soil]	23.6	38.3	21.5
CEC [$cmol^+/kg$]	3.0	9.0	3.7
pH (H_2O)	6.5	8-1	6.6
pH ($CaCl_2$)	5.9	7.2	6.1
Max. water holding capacity [g/100g dry soil]	24.1	29.0	26.9

B. STUDY DESIGN

1. Experimental conditions

The test substance was applied as 500 µg/mL solution in acetone in small doses of 100 µL (in total 0.750 mL) to 1.5 kg (dry-weight equivalent) of soil bulk mass, while homogenising the soil with a hand-hold mixer between dosing. After 5 minutes of homogenisation, the water content was measured / re-adjusted gravimetrically. The application rate (based on dry soil weight) of Reg. No. 89517 dosed to bulk soil was 0.25 mg/kg. Assuming a soil depth of 2.5 cm and a soil density of 1.5 g/cm^3 this corresponds to a theoretical field application rate of about 100 g/ha.

50 g of dry soil mass equivalents were filled in incubation vessels of 1000 mL and loosely covered with a plug of paper tissue to prevent excessive loss of water and to allow exchange of air and then incubated at 20 ± 1°C in a thermo stated cabinet in the dark.

2. Sampling

Duplicate soil samples were taken at 0, 2, 7, 15, 18, 21, 30, 45 (DAT) for soil Lufa 2.3, at 0, 2, 7, 15, 18, 21, 30 days after treatment (DAT) for soil Lufa 5M, and at 0, 2, 7, 15, 21, 30, 59 (DAT) for soil Li10.

3. Description of analytical procedures

For the determination of residues of Reg. No. 89517, portions of 10.0 g soil sample (based on dry mass) were extracted one time with 25 mL of acetonitrile/water (1/1 v/v, 0.1% formic acid) and two more times with 25 mL of acetonitrile containing 0.1% formic acid. The extracts were combined and diluted to exactly 100 mL with acetonitrile/water (1/1 v/v, 0.1% formic acid). An aliquot of the final extract was diluted by a factor DF of 10 with acetonitrile/water (1/1 v/v, 0.1% formic acid) and injected for LC-MS/MS analysis.
The analytical method was validated and achieved a limit of quantitation (LOQ) of 0.005 mg/kg and a limit of detection (LOD) of 0.001 mg/kg for Reg. No. 89517.

For all three soils, results of method validation showed an average recovery from 86 % to 102 % at a fortification level of 0.25 mg/kg and from 85 % to 98 % at a fortification level of 0.0025 mg/kg.

4. Calculation of the degradation rate

The degradation rate constant (k) of Reg. No. 89517 in soil was determined by fitting the soil residues using the software package KinGUI version 2.2.

II. RESULTS AND DISCUSSION

A. TRANSFORMATION OF PARENT COMPOUND

Residues of the analyte extracted after given incubation periods are summarised in Table 7.1.2.1.2-18 to Table 7.1.2.1.2-20. The analyte concentration in the soils generally decreased in all soils in the scope of the experiment from 91 to 102% of applied at 0 DAT to 1.9 to 5.7% of applied at the end of the incubation period after 30 to 59 days.

Table 7.1.2.1.2-18: Residues in soil Lufa 2.3 after treatment with Reg. No. 89517 and incubation under aerobic conditions

	Residues [mg/kg]	Residues [% of applied residue]
0	0.299	91
0	0.298	90
2	0.295	89
2	0.295	89
7	0.192	58
7	0.182	55
15	0.084	26
15	0.085	26
18	0.066	20
18	0.061	18
21	0.039	12
21	0.040	12
30	0.019	5.9
30	0.018	5.5
45	0.010	3.1
45	0.010	3.0

DAT = days after treatment
ERR = extractable residues

Table 7.1.2.1.2-19: Residues in soil Li10 after treatment with Reg. No. 89517 and incubation under aerobic conditions

	Residues [mg/kg]	Residues [% of applied residue]
0	0.243	102
0	0.249	104
2	0.223	94
2	0.232	97
7	0.163	68
7	0.160	67
15	0.103	43
15	0.101	42
21	0.063	26
21	0.067	28
30	0.034	14
30	0.033	14
59	0.014	5.8
59	0.014	5.7

DAT = days after treatment
ERR = extractable residues

Table 7.1.2.1.2-20: Residues in soil Lufa 5M after treatment with Reg. No. 89517 and incubation under aerobic conditions

	Residues [mg/kg]	Residues [% of applied residue]
0	0.243	97
0	0.252	100
2	0.184	73
2	0.193	77
7	0.096	38
7	0.067	27
15	0.013	5.2
15	0.014	5.7
18	0.008	3.0
18	0.010	4.0
21	0.009	3.4
21	0.006	2.6
30	0.005	1.8
30	0.005	1.9

DAT = days after treatment
ERR = extractable residues

B. KINETIC ANALYSES

Reg. No. 89517 soil residues in µg/kg were used for kinetic modelling calculations using KinGUI version 2.2. SFO kinetics was the best-fit model for all three soils and gave an acceptable description of the data statistically as well as visually, resulting in trigger endpoints for DT_{50} of 8.03 days for LUFA 2.3, 4.22 days for LUFA 5M and 11.12 days for Li 10.

III. CONCLUSION

This study demonstrates that Reg. No. 89517 was degraded in all three soils used in the study. Residues of Reg. No. 89517 observed in soils were fitted using the software package KinGUI version 2.2. SFO kinetics was the best-fit model for all three soils and showed acceptable fits to the measured data statistically as well as visually. The resulting trigger endpoints DT_{50} for Reg. No. 89517 were as follows: 8.03 days for soil LUFA 2.3, 4.22 days for soil LUFA 5M and 11.12 days for soil Li 10. The modelling endpoints derived from modelling results are the same as trigger endpoints.

Supplementary information

A potential metabolite that can be cleaved off from RP 30228 is the hydantoin moiety with the code RP 30181 (3-isopropyl-2,4-dioxo-imidazolidine) leaving dichloroaniline from the labelled part of the molecule. But degradation can also occur via ring open metabolites such as RP 36221 or RP 35606 so that the formation of larger amounts of RP 30181 is not likely. As 2,4-dioxo-imidazolidine (hydantoin) is a known metabolite of metiram and data on the degradation in soil are available, these data are presented to substitute information for RP 30181. The following study shows that hydantoin is rapidly degraded in soil (DT_{50} below 1 day) and it is expected that the half-life of RP 30181 would be in a similar range. So a concern to the environment from this potential metabolite is not expected.

Report:	CA 7.1.2.1.2/4 Class T., 2010a Ethylene Urea and Hydantoin (metabolites of Metiram, BAS 222 F): Study on aerobic soil degradation 2010/1056131
Guidelines:	OECD 307 (2002), BBA IV 4-1, EPA 835.4100, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Umweltministerium Baden-Wuerttemberg, Stuttgart)

Executive Summary

The objective of the study was to examine the aerobic degradation of hydantoin (HY) in three different soils: LUFA 2.2 (loamy sand), Li 10 (loamy sand), and Bruch West (sandy loam). The treated soils were incubated at 45% maximum water holding capacity (MWHC) at 20°C in the dark. The application rate (based on dry soil weight) of the test item dosed to bulk soils was 0.8 mg/kg. Assuming a soil depth of 2.5 cm and a density of 1.5 g/cm^3 this rate corresponds to a theoretical field application rate of 0.3 kg/ha for hydantoin.

After given incubation periods, replicate soil aliquots were collected and extracted with methanol/water for subsequent LC/MS/MS determination. The method was validated with acceptable average recoveries in a range of 87 % to 105 % (relative standard deviations RSD always < 20 %). The method achieved a limit of quantitation (LOQ) of 0.04 mg/kg for hydantoin (i.e. 5 % of the respective dose level).
The evaluation of the aerobic degradation of hydantoin applying FOCUS kinetics analyses and single first order (SFO) kinetics resulted in degradation times DT_{50} for hydantoin of about 0.1 to 0.3 days.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item Imidazolidine-2,4-dione, hydantoin
Lot No.: S23814
Purity: 98%
Molecular weight: 100.1 g/mol

2. Soils

The study was conducted with three different soils from Germany. Soils were acclimatised immediately at room temperature in the dark with soil moistures adjusted to 45 % of their respective maximum water holding capacities. The physico-chemical characterisation of the soils (determined at BioChem Laboratory) is provided in Table 7.1.2.1.2-21.

Table 7.1.2.1.2-21: Soil characteristics

Soil designation Origin	**LUFA 2.2**	**Li 10**	**Bruch West**
Textural class (German scheme, DIN 4220)	Silt sand	Loamy sand	Loamy sand
Soil texture [%], (German scheme)			
Sand	83.4	80.7	62.9
Silt	12.4	13.8	25.4
Clay	4.2	5.5	11.7
Textural class (USDA scheme)	Loamy sand	Loamy sand	Sandy loam
Soil texture [%], (USDA scheme)			
Sand	84.0	81.4	65.7
Silt	11.8	13.2	22.6
Clay	4.2	5.5	11.7
Microbial biomass [mg C/kg dry soil]	42.5	20.6	29.4
Organic carbon [%]	1.95	0.86	1.37
CEC [$cmol^+/kg$]	6.4	4.1	10.1
pH ($CaCl_2$)	5.7	6.2	7.5
pH (water)	6.2	6.8	8.0

B. STUDY DESIGN

1. Experimental conditions

The application rate (based on dry soil weight) of the test item dosed to bulk soils was 0.8 mg/kg for hydantoin which is relatively difficult to analyse. Assuming a soil depth of 2.5 cm and a density of 1.5 g/cm^3 this rate corresponds to a theoretical field application rate of 0.3 kg/ha.

Hydantoin was applied as 0.80 mg/mL solution in acetonitrile, i.e. 1.08 to 1.14 mL were applied in small doses of 0.25 mL to about 1 kg (dry-weight equivalent) of soil bulk mass. The bulk soils were homogenised with a hand-hold mixer in between dosing.

25-g of dry soil mass equivalents was measured from the dosed homogenised soils into incubation flasks. Tare and gross weights of each flask were noted to allow for adjustment of soil moisture. The incubation flasks were incubated at 20±2 °C in a thermostated cabinet in the dark. Additionally, untreated bulk soil was kept in the thermostated cabinet, covered with a moistened towel to prevent excessive loss of water.
Treated soils were sampled at 0.25, 2.0, 6.0, 20, 24 and 28 hours (28 hours only for soil Li10) for hydantoin.

2. Description of analytical procedures

An API 5500 triple quadrupole LC-MS/MS system was used for the determination of hydantoin in soil extracts. For the hydantoin soil extraction, 25-g soil aliquots (W, based on dry soil weight) were extracted with 50 mL of methanol/water (1/1 v/v) by shaking mechanically at 300 rpm for about 30 min. Thereafter the sample was centrifuged and an aliquot of the supernatant transferred into an autosampler vial for LC-MS/MS analysis.

II. RESULTS AND DISCUSSION

A. Microbial biomass

The microbial biomass (was determined before application, and after 2 weeks resulting in the following values for microbial biomass verifying that the soil was viable throughout the incubation period:

Table 7.1.2.1.2-22: Microbial biomass

Soil Type	Microbial biomass 29 - Jan - 10 (treated soils) [mg C /100 g dry soil]	Microbial biomass 11 - Feb - 10 (treated soils) [mg C /100 g dry soil]
Li10	28	17
LUFA 2.2	25	29
Bruch West	26	21

B. LC-MS/MS Results

Table 7.1.2.1.2-23 gives the LC-MS/MS results for soil degradation. This table also shows the remaining analyte as percentage of the initially applied residue (%AR: nominally 0.8 mg/kg) at the beginning of the incubation.

Table 7.1.2.1.2-23: Degradation of hydantoin

Soil type	Soil LUFA 2.2		Soil Li10		Soil Bruch West	
[hours]	[mg/kg]	[%AR]	[mg/kg]	[%AR]	[mg/kg]	[%AR]
0.25	0.721	90.2	0.799	99.8	0.704	88.0
2.0	0.560	70.0	0.688	86.0	0.468	58.5
2.0	0.578	72.3	0.686	85.8	0.478	59.8
6.0	0.310	38.8	0.612	76.5	0.206	25.8
6.0	0.318	39.8	0.608	76.0	0.212	26.5
20	<LOQ	<LOQ	0.083	10.4	<LOQ	<LOQ
20	<LOQ	<LOQ	0.079	9.9	<LOO	<LOO
24	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
24	<LOQ		<LOQ		<LOQ	<LOQ
28	-	-	<LOQ	<LOQ	-	-
28	-	-	<LOQ		-	-

B. Kinetic Analyses

The residues observed for the analytes (expressed as %AR) in the incubated soil samples were fitted using the software package KinGUI version 1.1. The kinetic analyses were interpreted as recommended in Chapter 7.1.2 of FOCUS [*FOCUS (2006)*] to derive modelling endpoints.

The derived modelling endpoints are as follows:

Table 7.1.2.1.2-24: Hydantoin: DT_{50} and DT_{90} values

Substance, Soil Type, Model	DT_{50} [hours]	DT_{90} [hours]	DT_{50} [days]	DT_{90} [days]	$\chi 2$ error [%]	k ± St.dev.	M0 ± St.dev.	Type I error (Prob. > t)
EU, LUFA 2.2, SFO	4.7	15.7	0.20	0.65	1.7	0.1469 ±0.0050	90.5±0.7	<0.001
EU, Li 10, SFO··	7.8	25.8	0.33	1.08	10.1	0.0894 ±0.0100	101.6±2.7	<0.001
EU, Bruch West, SFO	3.2	10.7	0.13	0.45	1.2	0.2148 ±0.0063	87.8±0.6	<0.001

III. CONCLUSION

The objective of the study was to examine aerobic degradation of hydantoin in three different soils.
The evaluation of the aerobic degradation of hydantoin applying FOCUS kinetics analyses and single first order (SFO) kinetics resulted in degradation times DT_{50} for hydantoin of about 0.1 to 0.3 days.

Report:	CA 7.1.2.1.2/5 Szegedi K., 2014a Update of the kinetic evaluations of older soil degradation studies with BAS 610 F (Iprodione) and DCA (3,5-Dichloroaniline) according to the FOCUS kinetics guideline 2013/1311394
Guidelines:	FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011
GLP:	no

Summarised in chapter CA 7.1.2.1.1, CA 7.1.2.1.1/3.

Overall Summary on Rate of Degradation in Soil

An overview on the laboratory half-lives of the metabolites of iprodione at 20°C is provided in Table 7.1.2.1.2-25.

The following considerations need to be taken into account for the endpoint selection:

RP 25040
The degradation of RP 25040 was investigated in three soils where RP 25040 was applied [*Class – 2013/1078064*]. Normalised $DegT_{50}$ values derived from this study were between 0.75 and 4.1 days. Furthermore, RP 25040 was observed in the aerobic soil degradation study with iprodione [*Hartman et al. (2014a) – 2013/1240319, CA 7.1.2.1.1/1*] and kinetic evaluation [*Budde (2014b) – 2013/1311391, CA 7.1.2.1.1/2*] resulted in normalised $DegT_{50}$ values between 5.3 and 19.2 days in three soils, with formation fractions between 0.15 and 0.19. For the fourth soil, no statistically significant degradation rate could be determined, as in this soil, very little RP 25040 (<1% AR) was formed and residues of RP 25040 showed no clear pattern of decline. This last soil was not considered for selection of a modelling endpoint, as inclusion of a default value (i.e. 1000 days) is not justified: i) the value of 1000 days is clearly outside the range of the values determined in other soils; ii) the value is clearly outside the range of the values determined in other studies in which the metabolite was applied; iii) an almost linear relationship between pH and degradation rate of RP 25040 can be noted, with $DegT_{50}$ values < 2 days at higher pH values. It is therefore deduced that the low degradation rate of RP 25040 in soil LUFA 5M] was an artefact due to the low amounts of RP 25040 formed (<1% TAR). For the PEC_{gw} calculations of RP 25040, the arithmetic mean formation fraction of 0.17 was taken into account. To cover observed degradation behaviour, PEC_{gw} calculations will be performed using both the longest $DegT_{50}$ of 19.2 days and the shortest $DegT_{50}$ of 0.75 days. For PEC_{soil} and PEC_{sw} calculations worst case $DegT_{50}$ of 19.2 days will be used.

RP 35606

For RP 35606, normalised $DegT_{50}$ values between 2.1 and 6.7 days were derived during kinetic evaluation [*Budde (2014b) – 2013/1311391, CA 7.1.2.1.1/2*] of the aerobic soil degradation study [*Hartman et al. (2014a) – 2013/1240319, CA 7.1.2.1.1/1*]. The geometric mean $DegT_{50}$ value of 3.26 days will be used for the PEC_{gw} calculations, together with the arithmetic mean formation fraction of 0.73 at Tier 1. Additionally, Tier 2 calculations were performed for acidic soils, using a formation fraction of 0.52 relevant for acidic soils.

RP 30228

For RP 30228 (formed via intermediate RP 35606), normalised $DegT_{50}$ values between 14.6 and 66.3 days were derived during kinetic evaluation [*Budde (2014b) – 2013/1311391, CA 7.1.2.1.1/2*] of the aerobic soil degradation study [*Hartman et al. (2014a) – 2013/1240319, CA 7.1.2.1.1/1*]. The geometric mean $DegT_{50}$ value of 35.7 days was used for the PEC_{gw} calculations. Calculated formation fractions were between 0.21 and 0.92, and calculations were performed with the arithmetic mean (n=4) formation fraction of 0.41.

RP 36221

RP 36221 (second degradation product of RP 35606) was observed in the aerobic soil degradation study [*Hartman et al. (2014a) – 2013/1240319, CA 7.1.2.1.1/1*], and kinetic evaluation [*Budde (2014b) – 2013/1311391, CA 7.1.2.1.1/2*] resulted in a normalised $DegT_{50}$ value of 154.2 in one soil (with a pH of 5.5), while in the other three soils, no reliable degradation rate could be determined. The degradation of RP 36221 was additionally investigated in three soils where RP 36221 was applied [*Class (2013a) – 2013/1211061, CA 7.1.2.1.2/1*]. The $DegT_{50}$ value derived from this study was 715 days in one soil (pH 7.4), while for the other two soils, no reliable degradation rate could be determined. Calculations were performed for all FOCUS scenarios with two modelling endpoints: a worst-case $DegT_{50}$ value of 1000 days was assumed for the PEC_{gw} calculations for soils with a pH>5.5, while for acidic soils (pH≤5.5), the $DegT_{50}$ of 154.2 days was selected as relevant.

Calculated formation fractions for RP 36221 [*Budde (2014b) – 2013/1311391, CA 7.1.2.1.1/2*] were between 0.07 and 0.15 for three soils (pH<7) and could not be calculated for the fourth soil (pH 7.3). For this latter soil, a worst-case formation fraction of 1 was assumed, and the arithmetic mean (n=4) formation fraction of 0.33 was used for the calculations.

DCA

DCA was observed in the aerobic soil degradation study [*Hartman et al. (2014a) – 2013/1240319, CA 7.1.2.1.1/1*], and kinetic evaluation [*Budde (2014b) – 2013/1311391, CA 7.1.2.1.1/2*] resulted in normalised $DegT_{50}$ values between 10.0 and 32.2 days in three soils. For the fourth soil, no statistically significant degradation rate could be determined, as in this soil, residues of DCA showed no clear pattern of decline. This soil was not considered for selection of a modelling endpoint, as inclusion of a default value (i.e. 1000 days) is not justified: i) the value of 1000 days is clearly outside the range of the values determined in other soils; ii) the value is clearly outside the range of the values determined in other studies in which the metabolite was applied. The degradation of DCA was additionally investigated in three soils where DCA was applied [*Gouot (1981) – 1981/1000805; Quarmby (2000) – B002966*]. Kinetic evaluation [*Szegedi (2014) – 2013/1311394, CA 7.1.2.1.1/3*] yielded normalised $DegT_{50}$ values between 22.6 and 57.2 days. The geometric mean $DegT_{50}$ value of 30.3 days (n=6) was used for the PEC_{gw} calculations. As a worst-case assumption, the formation fraction of DCA from each of the three precursors was set to 1.

Table 7.1.2.1.2-25: Degradation rates of the metabolites of iprodione in soil under aerobic conditions in the laboratory

Soil	pH_{CaCl2} [-]	Best-fit model	Trigger-$DegT_{50}$ [d]	Modelling-$DegT_{50}$ [d]	Formation fraction [-]	Study / Kinetic evaluation
RP 25040						
LUFA 2.2	5.5	SFO	22.5	19.2	0.17	Hartman et al. (2013/1240319) / Budde (2013/1311391)
Li 10	6.2	SFO	12.0	11.2	0.19	
LUFA 2.3	6.7	SFO	6.9	5.3	0.15	
LUFA 5M	7.3	SFO	-[a]	-[c]	0.01	
Li10	6.3	FOMC	2.8	4.1	n/a	Class (2013/1078064)
LUFA 5M	7.4	FOMC	0.38	0.75	n/a	
LUFA 2.3	7.0	FOMC	0.44	1.7	n/a	
RP 36221						
LUFA 2.2	5.5	SFO	180.4	154.2	0.15	Hartman et al. (2013/1240319) / Budde (2013/1311391)
Li 10	6.2	SFO	-[a]	1000[b]	0.07	
LUFA 2.3	6.7	SFO	-[a]	1000[b]	0.08	
LUFA 5M	7.3	SFO	-[a]	1000[b]	-[a]	
Li10	6.3	SFO	-[a]	1000[b]	n/a	Class (2013/1211061)
LUFA 5M	7.4	SFO	714.5	714.5	n/a	
LUFA 2.3	7.4	SFO	-[a]	1000[b]	n/a	
RP 30228						
LUFA 2.2	5.5	SFO	43.1	36.8	0.21	Hartman et al. (2013/1240319) / Budde (2013/1311391)
Li 10	6.2	SFO	48.7	45.6	0.28	
LUFA 2.3	6.7	SFO	86.1	66.3	0.22	
LUFA 5M	7.3	SFO	22.7	14.6	0.92	
RP 35606						
LUFA 2.2	5.5	SFO	3.2	2.7	0.52	Hartman et al. (2013/1240319) / Budde (2013/1311391)
Li 10	6.2	SFO	3.2	3.0	0.82	
LUFA 2.3	6.7	SFO	2.7	2.1	0.85	
LUFA 5M	7.3	SFO	10.4	6.7	0.76	
DCA						
LUFA 2.2	5.5	SFO	37.7	32.2	1 / -[a] / -[a,d]	Hartman et al. (2013/1240319) / Budde (2013/1311391)
Li 10	6.2	SFO	-[a]	-[c]	-[a] / -[a] / -[a,d]	
LUFA 2.3	6.7	SFO	40.3	31.0	-[a] / -[a] / -[a,d]	
LUFA 5M	7.3	SFO	15.5	10.0	-[a] / 0.54 / -[a,d]	
'Sandy Loam'	6.3[e]	FOMC	8.6	57.2	n/a	Gouot (1981/1000805) / Szegedi (2013/1311394)
Columbia / Silt loam	6.9[f]	FOMC	2.7	22.6	n/a	Quarmby (B002966) / Szegedi (2013/1311394)
Modera / Sandy loam	7.3[f]	FOMC	8.6	59.6	n/a	

[a] no reliable parameter could be determined
[b] default value of 1000 d used as modelling-DT_{50}
[c] no degradation observed in this soil, not considered for derivation of modelling endpoints
[d] from RP 25040 / RP 30228 / RP 36221
[e] determined in KCl
[f] determined in water (1:1)
n.p. not performed
n/a not applicable (metabolite was applied)

CA 7.1.2.1.3 Anaerobic degradation of the active substance

Report:	CA 7.1.2.1.3/1 Sopena-Vazquez F.,Bayer H., 2014a Anaerobic soil metabolism of BAS 610 F (Iprodione) 2013/1283134
Guidelines:	OECD 307, EPA 835.4200
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

The report on the fate of iprodione in anaerobic soil is summarised in CA 7.1.1.2/1. The calculated degradation rates for iprodione can be found there.

CA 7.1.2.1.4 Anaerobic degradation of metabolites, breakdown and reaction products

Report: Unsworth, R.H. & Clarke, D.E. (1999): [14C]-RP030228: Anaerobic soil degradation (study no 16828).
Report Rhône-Poulenc Agriculture LTD no 202076 of December 2, 1999.
C022378
GLP: Yes

This study was carried out to elucidate the rate and route of [^{14}]C-RP030228 degradation under anaerobic conditions. The soil selected was a sandy loam soil similar to that used to study the rate of RP 30228 degradation under aerobic conditions.

Table 7.1.2.1.4-1: Soil characteristics

Soil reference	**99/01**
Location	Aldhams Farm, Dead Lane, Lawford, Manningtree, Essex, UK
OS map reference	TL562046
Particle size analysis Coarse/very coarse sand (500 µm-2mm) Medium sand (250 µm-500 µm) Fine sand (106 µm – 250 µm) Very fine sand (63 µm – 106 µm) Coarse silt (20 µm – 63 µm) Fine silt (2 µm – 20 µm) Clay (< 2µm) *Total*	% 6.97 15.44 27.16 8.76 18.38 13.31 9.99 *100.01*
Textural class ADAS (USDA)	 Sandy loam (sandy loam)
Organic carbon	3.6 %
Organic matter	6.2 %
pH Deionised water 0.01M Calcium chloride 1M Potassium chloride	 6.3 5.6 5.3
Water content (before acclimatisation) Atmospheric pressure	% 31.4
Water holding capacity Maximum @ atmospheric pressure	% 59.2
Cation exchange capacity	8.3 mMeq/100g
Microbial biomass Initial Final	 -7 µgCg^{-1} soil 47 µgCg^{-1} soil

Portions (100 g oven dry equivalent) of sandy loam soil were flooded with deionised water and purged with nitrogen for 33 days prior to treatment to establish anaerobic conditions. A solution of [^{14}C]-RP030228 in a minimal volume of acetonitrile was applied to the water surface at an application rate equivalent to 964 g a.s./ha.

During incubation the system was continually purged with nitrogen to maintain anaerobic conditions. The nitrogen was then passed through a single trap containing ethylene glycol and two further traps containing 2M aqueous potassium hydroxide to trap liberated volatile materials. Duplicate samples were removed from the system for analysis after 0, 1, 3, 7, 14, 28, 56 and 120 days incubation. At each sampling point the water was separated from the soil and the two phases were analysed separately. The radioactivity present in the water phase was quantified by Liquid Scintillation Counting (LSC) of representative aliquots. The water phase from each sample up to and including the day 14 timepoints, was analysed by Thin Layer Chromatography (TLC) with confirmatory High Performance Liquid Chromatography (HPLC) of representative samples.

All soil samples were extracted with acetonitrile and acetonitrile/water by shaking at room temperature, followed by Soxhlet extraction with acetonitrile/water, where necessary to extract > ca. 90% applied radioactivity. The radioactivity present in soil extracts was quantified by LSC of representative aliquots. The "cold" solvent extracts for each soil sample were combined and analysed by TLC, with confirmatory HPLC of representative samples. Soxhlet extracts were kept separate and analysed by TLC. Structural confirmation of the species present was obtained by liquid-chromatography mass spectrometry (LC-MS) of a representative soil extract concentrate. The soil was then air-dried, ground to a fine powder and the residual radioactivity quantified.

Results: RP 30228 dissipated rapidly from the water to the soil phase. After 7 days, only 12% applied radioactivity remained in the water. This declined to 1% applied radioactivity after 120 days incubation (see Table 7.1.2.1.4-2).

Table 7.1.2.1.4-2: Composition of radioactivity in water phase (by TLC)

Sampling intervals	**% Applied radioactivity**							
	0 hours		**1 day**		**3 days**		**7 days**	
Sample ID	4	5	10	28	13	23	6	29
RP 30228	**66.25**	**65.52**	**33.43***	**31.29***	**16.18***	**23.79***	**16.09***	**7.63***
Total	66.25	65.52	33.43	31.29	16.18	23.79	16.09	7.63
Sampling intervals	**14 days**		**28 days**		**56 days**		**120 days**	
Sample ID	22	30	15	24	8	18	16	25
RP 30228	**6.50**	**6.58**	**nt**	**nt**	**nt**	**nt**	**nt**	**nt**
Total	6.50	5.68	3.88	3.00	2.23	2.61	1.16	1.29

* Samples also analysed by HPLC to confirm that material present at the origin of the TLC plate was RP 30228

The DT_{50} and DT_{90} values (disappearance times for 50% and 90% of the initial material present) for RP 30228 in the water phase were calculated using first order kinetics; Timme-Frehse and KIM modelling program (Schering AG, version 1.0) are summarised in Table 7.1.2.1.4-3.

Table 7.1.2.1.4-3: DT_{50} and DT_{90} values for RP 30228 dissipation from the water phase

	Kinetics (days)					
	First-order		Timme-Frehse		KIM	
Phase	DT_{50}	DT_{90}	DT_{50}	DT_{90}	DT_{50}	DT_{90}
Water	24.35	80.87	0.36	9.83	0.42	8.03

The levels of RP 30228 in the soil phase reached a maximum of 100% after 56 days and remained at a constant level of 87% - 90% applied radioactivity for the 120-day duration of the study (see Table 7.1.2.1.4-4).

Table 7.1.2.1.4-4: Composition of radioactivity in soil phase (by TLC)

Sampling intervals	% Applied radioactivity							
	0 hours		1 day		3 days		7 days	
Sample ID	4	5	10	28	13	23	6	29
RP 30228	**29.05***	**30.52***	**59.08**	**61.20**	**75.45**	**73.56**	**81.76***	**88.41***
Total	29.05	30.52	59.08	61.20	75.75	73.56	81.76	88.41
Sampling intervals	**14 days**		**28 days**		**56 days**		**120 days**	
Sample ID	22	30	15	24	8	18	16	25
RP 30228	**87.47**	**91.41**	**89.27***	**89.07***	**86.29***	**100.04***	**90.63**	**87.70**
Total	87.47	91.41	89.27	89.07	86.29	100.04	90.63	87.70

* Samples also analysed by HPLC to confirm that material present at the origin of the TLC plate was RP 30228

Low levels of 2 minor metabolites each at < 3.5% applied radioactivity were detected from day 28 and day 56 sampling points only.
As no significant degradation of RP 30228 occurred in the soil phase it was not possible to calculate DT_{50} and DT_{90} values for this phase.

Low levels of volatile material accounting for < 0.1% applied radioactivity were detected at all sampling points up to and including 120 days.

The mean total radioactivity recovery was 100.8% applied radioactivity. Total radioactivity recoveries were within the acceptable range of 90-110% applied radioactivity for all timepoints.

Conclusion: RP 30228 is rapidly dissipated from the water to the soil but undergoes limited degradation in the entire system under anaerobic conditions at 20°C, in the absence of light. After 120 days the amount of RP 30228 remaining in the entire system was > 89% of applied radioactivity.

CA 7.1.2.2 Field studies

Numerous terrestrial field dissipation and soil residue studies were performed for iprodione. An overview is presented in Table 7.1.2.2-1.

Table 7.1.2.2-1: List of terrestrial field dissipation studies in soil performed with iprodione

Reference	Sites	Application rate [kg/ha]	Crop	Incubation period [days]	Remark
Norris (1991)	North Carolina California	8 x 1.1	Carrot		Not considered relevant
Walker et al. (1981)		3 x 5			Not considered relevant
Godward and Maycey (1981)	Canada	1 x 0.75	Turf		Not considered relevant
Maycey (1983)	Canada	2 x 0.3	Turf		Not considered relevant
Piznik et. al (1983) R003014	Louisiana	2 x 1.1	Rice plots		Field metabolism, not considered relevant
Gemma et al. (1983)	Louisiana Arkansas	2 x 0.56	Rice plots		Not considered relevant

These studies were not considered appropriate for the dossier as the studies were either conducted for non-representative uses like turf and rice and/or multiple applications were made to cropped fields, so that kinetic evaluation was difficult. The degradation of iprodione is enhanced by previous applications (Adaptation of soils, as described in the literature: A. Walker, 1987; Pestic. Sci. **21**, 219-231 and Pestic. Sci.; 1987, **21**, 235-240). The most appropriate study to derive persistence and modelling endpoints was conducted by Hardy [*Hardy (2001) – 2013/1211061, CA 7.1.2.2.1/1*] and is presented below. The kinetic evaluation of the study was performed according to the latest FOCUS kinetics guidance. To cover acidic soils a further study was conducted in France [*Richter & Kuhnke (2012) – 2011/1284995, CA 7.1.2.2.1/3*] and evaluated by Platz [*Platz (2011) – 201171252227, CA 7.1.2.2.1/5*] and Szegedi [*Szegedi (2011) – 2011/1285122, CA 7.1.2.2.1/4*].

CA 7.1.2.2.1 Soil dissipation studies

Report:	CA 7.1.2.2.1/1 Hardy I.A.J., 2001a Iprodione: Field soil dissipation study in Europe C015666
Guidelines:	EEC 95/36, EEC 91/414 Annex II 7.1.1.2.2
GLP:	Yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

The environmental behaviour of iprodione was studied in the field at four locations in Europe (Germany, France, UK and Spain). A single spray application was performed to bare soil in spring at a nominal rate of 4.0 kg a.s. ha^{-1} (WP formulation), immediately after the sowing of grass. Soil samples from different depths to a maximum of 60 cm were collected at regular intervals for up to one year and analysed for iprodione and its metabolite RP 30228.

Iprodione was rapidly degraded under field conditions in all four contrasting soils and climates. The mean DT_{50} for iprodione was 12.7 days and the corresponding mean DT_{90} was 42.2 days. Iprodione residues were less than 10% of applied within one month at Lyon and Manningtree, and within two months at Goch and Seville. Metabolite RP 30228 achieved a maximum residue of 22 % of applied parent at Seville.

Iprodione and RP 30228 were found to have low mobility under field conditions at the four sites in Europe despite precipitation plus irrigation exceeding average rainfall during the period of the study. At all sites the vast majority of the residues remained in the 0 to 10 cm layer. This field study demonstrates the low potential for unsaturated zone movement and negligible potential for iprodione and RP 30228 to appear in groundwater.

I. MATERIAL AND METHODS

A. MATERIALS

Iprodione:

Test item (formulation):	EXP01671H
Active ingredient:	Iprodione
Chemical name (IUPAC):	3-(3,5-Dichlorophenyl)-N-isopropyl-2,4-dioxoimidazolidine-1-carboxamide
Molar mass:	330.2 g/mol
CAS No.:	36734-19-7

Metabolite RP 30228:

Chemical name (IUPAC):	N-(3,5-dichlorophenyl)-3-isopropyl-2,4-dioxoimidazolidine-1-carboxamide
Molar mass:	330.2 g/mol
CAS No.:	63637-89-8

B. STUDY DESIGN

The behaviour of iprodione and its metabolite RP 30228 was investigated under field conditions at four locations for up to 24 months. Two sites were located in northern Europe, one in Germany (Goch-Nierswalde) and one in the UK (Manningtree). The other two sites were located in southern Europe, one in Spain (Seville) and one in southern France (Lyon). Iprodione was not used at any of the trial sites during the three preceding years.

The soil characteristics of the soils are presented in Table 7.1.2.2.1-1.

Table 7.1.2.2.1-1: Soil characteristics of the trial sites

Trial	Goch		Lyon	
Location	**Goch-Nierswalde, Germany**		**Pusignan, France**	
Soil properties	**0 - 30 cm**	**30 - 60 cm**	**0 - 30 cm**	**30 – 60 cm**
Soil class (ADAS)	Sandy silt loam	Sandy silt loam	Sandy silt loam	Clay loam
Clay [%]	10	11	14	21
Silt [%]	69	68	42	44
Sand [%]	22	21	44	35
Soil class (USDA)	Silt loam	Silt loam	Sandy loam	Loam
Clay [%]	10	11	14	21
Silt [%]	54	53	33	34
Sand [%]	37	36	53	45
Organic carbon [%]	2.0	0.4	0.5	0.4
pH ($CaCl_2$)	6.1	5.8	6.9	7.0
pH (H_2O)	6.4	6.3	7.1	7.3
pH (KCl)	5.9		6.8	6.7
CEC [mEq /100g]	26.3	19.1	9.7	12.5
WHC [%] *	56.9 / 58.1		56.7 / 45.2	
Biomass [µg C/g soil] *	331 / 355		80 / 186	
Trial	**Manningtree**		**Seville**	
Location	**Essex, UK**		**Torre de la Reina, Spain**	
Soil properties	**0 - 30 cm**	**30 - 60 cm**	**0 - 30 cm**	**30 – 60 cm**
Soil class (ADAS)	Sandy silt loam	Sandy silt loam	Sandy silt loam	Sandy loam
Clay [%]	11	12	12	12
Silt [%]	41	40	42	35
Sand [%]	48	48	46	53
Soil class (USDA)	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Clay [%]	11	12	12	12
Silt [%]	33	33	36	30
Sand [%]	56	56	53	58
Organic carbon [%]	1.1	0.4	0.7	0.8
pH ($CaCl_2$)	6.4	6.0	7.0	6.8
pH (H_2O)	6.6	6.5	7.4	7.2
pH (KCl)	6.0	5.4	6.6	5.9
CEC [$cmol^+$ /kg]	10.4	11.9	11.7	13.9
WHC [%] *	47.1 / 36.3		50.0 / 56.7	
Biomass [µg C/g soil] *	125 / 200		205 / 269	

CEC = effective cation exchange capacity
WHC = water holding capacity
* determined at the start of the study / one year after application (from 0-10 cm soil cores)

3. Experimental treatments

The experimental formulation EXP01671H was sprayed at all sites onto bare ground pre-emergence of grass. The formulation was a wettable powder containing 500 g kg^{-1} iprodione. The nominal dose rate was a single application of 4.0 kg a.s. ha^{-1}.

Rainfall was supplemented with irrigation to maintain the crop and achieve a total precipitation above the average precipitation on a monthly basis. Irrigation was generally stopped during the winter months, in accordance with the local practice. The plots were maintained relatively weed-free.

Application rates were confirmed both by analysing the content of filter papers placed on the soil and by analysing soil samples taken immediately after application. Application details are presented in Table 7.1.2.2.1-2.

Table 7.1.2.2.1-2: Application parameters of field trial sites treated with iprodione

Trial / Country	No. of applications	Application method	Application rate per treatment					No. of treated replicates	Application date
			Nominal [g a.s./ha]	Subplot	Calibrated app. rate * [g a.s./ha]	Dose verification**	Dose verification ** (mean % of calibrated app. rate)		
Goch Germany	1	Spray to bare soil	4000	1 2 3 4	4000	3200 3600 3400 3300	84	1 10 m x 160 m	4 April 2000
Lyon France	1	Spray to bare soil	4000	1 2 3 4	4000	3600 3800 3500 3700	91	1 30 m x 37 m	16 May 2000
Manningtree UK	1	Spray to bare soil	4000	1 2 3 4	4000	3900 4100 4100 4000	101	1 12.5 m x 36 m	11 April 2000
Seville Spain	1	Spray to bare soil	4000	1 2 3 4	4000	2500 2800 2600 2800	67	1 12 mx 116 m	21 May1999

* determined by calculation of spray liquid applied
** determined using filter papers placed on the soil [g a.s./ha]

The weather data for the duration of the study were obtained from nearby weather stations. The following parameters were collected if available: daily air and soil temperatures, daily rainfall and daily evaporation or potential evapotranspiration (PET). A summary of weather data is presented in Table 7.1.2.2.1-3.

Table 7.1.2.2.1-3: Summary of weather data at each field trial site

Trial	**Goch**				**Trial**	**Lyon**			
Location	**Germany**				**Location**	**France**			
Climatic conditions	**T_{mean} Air [°C]**	**T_{mean} Soil [°C]**	**Rainfall [mm]**	**Total water* [mm]**	**Climatic conditions**	**T_{mean} Air [°C]**	**T_{mean} Soil [°C]**	**Rainfall [mm]**	**Total water* [mm]**
Month					**Month**				
Apr 00	10.3	9.8	52.2	52.2	May 00	17.7	18.4	56.0	56.0
May 00	14.8	14.9	111.2	111.2	Jun 00	20.2	22.1	29.4	89.4
Jun 00	15.9	16.6	87.6	87.6	Jul 00	19.6	22.0	55.8	65.8
Jul 00	15.5	16.6	108.2	108.2	Aug 00	22.4	23.7	82.2	82.2
Aug 00	17.4	17.9	63.4	63.4	Sep 00	18.3	19.5	84.0	94.0
Sep 00	15.8	15.8	95.8	95.8	Oct 00	13.1	13.4	164.2	164.2
Oct 00	11.1	11.9	110.0	110.0	Nov 00	9.2	8.7	91.6	91.6
Trial	**Manningtree**				**Trial**	**Seville**			
Location	**UK**				**Location**	**Spain**			
Climatic conditions	T_{mean} Air [°C]	T_{mean} Soil [°C]	Rainfall [mm]	Total water* [mm]	**Climatic conditions**	T_{mean} Air [°C]	T_{mean} Soil [°C]	Rainfall [mm]	Total water* [mm]
Month					**Month**				
Apr 00	8.5	8.1	85.2	85.2	May 99	20.4	20.0	17.8	73.8
May 00	12.7	12.4	114.2	114.2	Jun 99	24.6	26.1	1.2	85.2
Jun 00	15.3	15.2	63.2 **	119.2	Jul 99	26.2	28.0	0.0	100.0
Jul 00	15.3	15.3	65.2	93.2	Aug 99	25.4	28.6	1.0	93.0
Aug 00	17.6	16.8	112.2	112.2	Sep 99	21.7	24.5	47.4	67.4
Sep 00	15.7	14.7	63.8	63.8	Oct 99	18.1	19.5	294.0	314.0
Oct 00	10.9	11.0	150.4	150.4	Nov 99	10.7	12.3	7.2	87.2
					Dec 99	9.9	10.8	36.4	96.4
					Jan 00	6.8	8.0	31.0	91.0
					Feb 00	12.2	10.9	1.6	81.6
					Mar 00	14.3	14.5	33.6	69.6
					Apr 00	13.5	15.3	159.6	159.6
					May 00	19.8	21.4	74.4	74.4

* Total water = sum of rainfall and irrigation
** Underestimate due to missing data

4. Sampling

Soil samples were taken prior to treatment, within four hours of application (time 0), at 3 and 7 days and at approximately 0.5, 1, 2, 4, 6 months after application, thereafter every three months. Sampling continued until total residues remaining were less than 10% of the applied dose and no further useful information could be gained on dissipation or movement of residues.

After application, twenty soil cores were taken, five in each of the four subplots. At time 0, each soil core consisted of a single 0 - 10 cm increment. The five cores from each subplot were combined and thoroughly mixed. A subsample was transferred to a labelled bag and transferred to a freezer (ca. -20°C) within six hours after sampling. Only 0 - 10 cm increments were taken until the rainfall plus irrigation exceeded 25 mm since application, then samples were taken down to 60 cm.

The detailed sampling intervals are presented in Table 7.1.2.2.1-4.

Table 7.1.2.2.1-4: Summary of sampling intervals at each field trial site

Trial	Country	Sampling intervals [days after treatment]
Goch	Germany	0, 3, 7, 15, 30, 62, 122, 181
Lyon	France	0, 3, 7, 14, 32, 66, 126, 184
Manningtee	UK	0, 3, 8, 15, 30, 62, 122, 192
Seville	Spain	0, 3, 7, 17, 31, 61, 124, 185, 272, 367

5. Description of analytical procedure

Residues were extracted from soil with a water:acetonitrile, 30:70 v/v mixture in an Accelerated Solvent Extractor at a temperature of 50°C and pressure of 1885 psi. Iprodione and RP 30228 residues were determined by LC/MS/MS. The limit of quantification (LOQ) of iprodione and RP 30228 in soil was 0.005 mg kg^{-1}.

Laboratory spiked samples were analysed at various time intervals to verify that the soil samples did not deteriorate during storage.

The resulting residue decay rate versus days after application was evaluated by a pesticide dissipation ModelManager.

II. RESULTS AND DISCUSSION

A. METHOD VALIDATION

Recoveries were determined for iprodione and RP 30228 by fortification experiments with the field soils. The mean of recoveries for iprodione at six concentrations in the range LOQ to 500 times LOQ was 81% to 90%. For RP 30228, the mean of recoveries at three concentrations in the range LOQ to 50 times LOQ was 97% to 100%.

The analytical method was very sensitive in comparison to the relatively high application rate of 4.0 kg ha^{-1} which is equivalent to about 2.7 mg kg^{-1} in the soil for 10 cm depth increments. The LOQ was less than 0.2% of the initial residue.

B. APPLICATION VERIFICATION

The results from filter papers and immediate post-application soil samples were generally found to be close to the calibrated application rate. The soil results in g a.s. ha^{-1} were significantly higher than the calibrated rate at Manningtree probably due in part to differences in the soil density after shallow cultivation and planting grass seed. The lower than calibrated application rate indicated by the filter paper result at Seville was not confirmed by the soil results which were close to the nominal rate.

C. FINDINGS

The results of storage stability studies showed no significant degradation of either iprodione or RP 30228 up to the maximum storage times of 9 months for the soil from Lyon, 12 months for the soil from Seville and 15 months for the soils from Goch and Manningtree.

Since recoveries were generally within the acceptable range of 70% to 110%, the measured residue values were used without correction. The concentrations in wet soil were converted to dry weight values using the ratio of soil wet weight to dry weight. Residue levels in mg kg^{-1} were converted to residue rates in g a.s. ha^{-1}.

Residue values of iprodione and its metabolite RP 30228 in g/ha dry soil are presented in Table 7.1.2.2.1-5.

Table 7.1.2.2.1-5: Total residues of iprodione and metabolite RP 30228 under field conditions in soil summed up for all depths analysed

	Iprodione [g/ha]					RP 30228 [g/ha]				
Trial	**Goch, Germany**									
DAT	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Mean**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Mean**
0	5214	3985	5921	3910	4757	26	20	24	20	23
3	3997	4294	5256	5330	4719	33	31	41	44	37
7	4192	5176	4155	4265	4447	47	55	44	47	48
15	3298	4566	4456	4529	4212	51	62	55	57	56
30	845	746	1138	919	912	67	49	58	51	56
62	73	113	111	63	90	21	29	27	17	24
122	41	49	55	45	48	13	11	21	13	15
181	33	70	82	19	51	12	19	27	0	15
Trial	**Lyon, France**									
DAT	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Mean**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Mean**
0	4670	6004	3827	4038	4635	40	63	39	56	50
3	3999	4591	4034	2298	3730	64	104	66	63	74
7	3470	4982	3113	2251	3454	50	105	41	62	64
14	3114	4684	2310	1677	2946	157	361	104	173	199
32	296	186	184	105	193	145	274	289	273	245
66	42	51	61	48	50	66	141	121	101	107
126	14	14	14	11	13	35	62	52	127	69
184	14	9	11	0	9	29	16	27	52	31
Trial	**Manningtree, UK**									
DAT	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Mean**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Mean**
0	5970	4762	5792	6290	5703	68	44	62	78	63
3	4694	2465	5987	4287	4358	76	35	113	72	74
8	3164	1869	4293	2332	2915	113	59	156	93	105
15	1478	3696	1667	2370	2303	170	222	144	252	197
30	445	337	441	447	418	116	147	142	138	136
62	109	95	108	100	103	80	67	112	80	85
122	35	87	62	72	64	29	53	39	23	36
192	32	25	53	30	35	11	21	23	11	16
Trial	**Seville, Spain**									
DAT	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Mean**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Mean**
0	3513	4885	3595	3733	3931	ND	ND	ND	ND	ND
3	2427	3243	2069	2603	2585	179	167	I57	174	169
7	1523	1776	2109	1764	1793	572	548	575	528	556
17	1091	2057	1074	805	1257	626	898	669	785	744
31	833	355	476	220	471	913	973	720	806	853
61	139	95	98	46	94	743	660	714	404	630
124	18	30	23	23	23	287	389	254	254	296
185	12	11	14	11	12	197	112	269	128	177
272	22	13	0	9	11	156	83	34	72	86
367	0	11	0	13	6	77	61	52	56	61

DAT = days after treatment
N.D. = not determined

Iprodione and RP 30228 were found to have low mobility under field conditions at the four sites in Europe despite precipitation plus irrigation exceeding average rainfall during the period of the study. At all sites the vast majority of the residues remained in the 0 to 10 cm layer. No residues were found in the soil deeper than 10 cm, except for two sporadic results, one at the Lyon site and the other at the Seville site. At Goch and Manningtree, no residues above LOQ were found deeper than 30 cm and the sporadic residues at 20 to 30 cm were at or just above the LOQ.

Iprodione residues were less than 10% of applied within one month at Lyon and Manningtree, and within two months at Goch and Seville. RP 30228 achieved a maximum residue of 1% to 22% of applied parent at Goch and Seville, respectively, and then declined after one month at all sites. The overall levels of RP 30228 were much lower than for iprodione and did not persist in the environment.

The decline kinetics were determined using ModelManager. If the data input to the model included a time interval when the residue value was below the limit of quantification then a value equal to half the limit of quantification was used (4.5 g ha^{-1} = 0.003 mg kg^{-1} on a dry weight basis). A summary of the dissipation kinetics is shown in Table 7.1.2.2.1-6.

Table 7.1.2.2.1-6: Summary of iprodione degradation kinetics

Trial, Country	Kinetic model	r^2	DT_{50} [d]	DT_{90} [d]
Goch, Germany	SFO	0.89	19.0	63.1
Lyon, France	SFO	0.85	13.5	44.8
Manningtree, UK	SFO	0.90	9.6	32.0
Seville, Spain	SFO	0.93	8.7	29.0
		Overall mean	**12.7**	**42.2**

III. CONCLUSION

Iprodione was rapidly degraded under field conditions in all four contrasting soils and climates. The mean DT_{50} was 12.7 days (range 8.7 to 19.0 days) and the corresponding mean DT_{90} was 42.2 days (range 29.0 to 63.1 days). Iprodione residues were less than 10% of applied within one month at Lyon and Manningtree, and within two months at Goch and Seville.

Metabolite RP 30228 achieved a maximum residue of from 1 to 22% of applied parent at Goch and Seville, respectively, and then declined after one month at all sites. The overall levels of RP 30228 were much lower than for iprodione which implies the conversion to RP 30228 is a minor route for degradation of iprodione.

Iprodione and RP 30228 were found to have low mobility under field conditions at the four sites in Europe despite precipitation plus irrigation exceeding average rainfall during the period of the study. At all sites the vast majority of the residues remained in the 0 to 10 cm layer. This field study demonstrated the low potential for unsaturated zone movement and negligible potential for iprodione and RP 30228 to appear in groundwater.

Report: CA 7.1.2.2.1/2
Budde E., 2014c
Kinetic evaluation of four field dissipation trails with BAS 610 F - Iprodione to obtain DegT50 in soil
2013/1311393
Guidelines: FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011, EFSA Guidance to obtain DegT50 values in soil (2010)
GLP: no

Executive Summary

The dissipation behaviour of BAS 610 F – iprodione in soil has been investigated in a field dissipation study including four field trials at four locations in Europe (Germany, France, UK and Spain), considering a range of different soils and climatic conditions. The purpose of this evaluation was to analyse the dissipation behaviour of iprodione in four soils under different climatic conditions and to derive normalised modelling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics. As photodegradation has been reported for iprodione, the recommendations provided in the EFSA opinion on estimating $DegT_{50}$ values in soil for modelling purposes [*EFSA (2010)*] were followed to exclude potential influence of surface processes and to obtain $DegT_{50}$ values in soil for modelling purposes.

Data derived from the field trials were normalised to reference conditions (20°C, pF2) prior to kinetic evaluation by time-step normalisation. Kinetic evaluation was performed on the normalised datasets in order to derive degradation parameters that are valid as modelling endpoints. The normalised field half-lives ($DegT_{50}$) for iprodione ranged from 3.5 to 11.5 days.

I. MATERIAL AND METHODS

The kinetic evaluation was conducted for four field trials with iprodione from the data of one field dissipation study, which can be found in CA 7.1.2.2.1/1 [*Hardy (2001) – 2001/1023435*]. The trials were situated at four locations in Europe (Germany, France, UK and Spain). A single spray application was performed to bare soil in spring at a nominal rate of 4.0 kg a.s. ha^{-1} (WP formulation), immediately after the sowing of grass. Rainfall was supplemented with irrigation to maintain the crop and achieve a total precipitation above the average precipitation on a monthly basis. Detailed soil characteristics as well as detailed sampling regime and analytical procedures are reported in the cited study.

Normalisation procedure

Evaluation of the suitability of field dissipation data for normalisation was performed according to the evaluation criteria for normalisation compiled by the Dutch regulatory authority (CTB criteria). As grass was grown during the trials, the influence of the crop had to be assessed. It was concluded that plant uptake had no influence on the behaviour of iprodione in the trials, due to the following reasons:

- no substantial amount of biomass could be formed during the rapid degradation of iprodione
- due to high K_{oc} of iprodione its bioavailability is limited
- the results of the kinetic evaluation showed that the degradation of iprodione can be described for all trials with the SFO kinetic model. Moreover, the fit quality is quite good considering general experience on fit quality for field studies. This is a clear indicator that the behaviour of iprodione in soil was governed by degradation as only dissipation pathway.

Temperature correction factors (f_{temp}) were determined to account for differences between actual daily temperatures as calculated by FOCUS-PEARL 4.4.4 or FOCUS-PEARL 2.2.2 and a reference temperature of 20°C using the Q_{10} approach as described in the report of the FOCUS soil modelling working group [see *FOCUS (1997): Soil persistence models and EU registration. The final report of the work of the Soil Modelling Work group of FOCUS (FOrum for the Co-ordination of pesticide fate models and their Use)*]. The Q_{10} response function was applied for temperatures above 0°C (see Equation 7.1.2.2.1-1 c). Below field temperatures of 0°C it was assumed that no degradation occurs (Equation 7.1.2.2.1-1 c). For the evaluation, the EFSA opinion on the default Q_{10} value [see *EFSA (2007): Scientific Opinion of the Panel on Plant Protection Products and their Residues on a request from EFSA related to the default Q10 value used to describe the temperature effect on transformation rates of pesticides in soil. The EFSA Journal (2007) 622, 1-32.*] was followed and 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 and the reference soil moisture (θ_{ref}) (Equation 7.1.2.2.1-1 d).

The normalised day lengths were derived according to Equation 7.1.2.2.1-1 a. For DAT 0, no normalisation was considered and application was assumed to occur at the time point zero. Normalised sampling days (DAT_{norm}) after application were calculated by cumulatively summing up normalised day lengths according to Equation 7.1.2.2.1-1 b.

Equation 7.1.2.2.1-1 Calculation of normalised 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} =	Normalised 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) $$f_{moist} = \begin{cases} \left(\frac{\theta_{act}}{\theta_{ref}}\right)^B & \text{for } \theta_{ref} > \theta_{act} \\ 1 & \text{for } \theta_{ref} \leq \theta_{act} \end{cases}$$

with:

D_{norm} =	normalised 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)	[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)	[-]
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	[-]

Table 7.1.2.2.1-7 shows the field sampling dates for the trial locations and the normalised (20°C, pF2) day lengths based on soil moisture and soil temperature data as simulated by FOCUS-PEARL.

Table 7.1.2.2.1-7: Time-step normalised (temperature and moisture) sampling days

Goch, Germany		Lyon, France	
DAT	**D_{norm}**	**DAT**	**D_{norm}**
0	0	0	0
3	1	3	2.6
7	2.2	7	4.9
15	4.8	14	10.1
30	12.5	32	28
62	33.1	66	61.7
122	77.5	126	130.2
181	121.3	184	162.9
Manningtree, UK		**Seville, Spain**	
DAT	**D_{norm}**	**DAT**	**D_{norm}**
0	0	0	0
3	0.8	3	3.9
8	2.3	7	9.4
15	4.9	17	21.8
30	11.4	31	44.1
62	28.3	61	98.7
122	68.5	124	196.1
192	109.7	185	242.6
-	-	272	274.5
-	-	367	333.7

As photodegradation has been reported for iprodione, the EFSA scientific opinion [*EFSA (2010): Guidance for evaluating laboratory and field dissipation studies to obtain DegT50 values of plant protection products in soil. EFSA Journal 8(12):1936:1-67*] was followed to exclude potential soil surface processes.

The field studies were assessed in regard to an appropriate number of data points for a kinetic assessment if samplings before 10 mm of cumulative precipitation were excluded. Five to ten data points remained for the kinetic evaluation of the field trials according to the EFSA opinion (see Table 7.1.2.2.1-8).

Table 7.1.2.2.1-8: Summary of evaluable field trials according to EFSA

Trial site	Country	Day of 10 mm rain (DAT)	Amount of rain until 1st considered data point [mm]	Remaining sampling days
Goch	Germany	9	16.0	5
Lyon	France	10	18.8	5
Manningtree	United Kingdom	2	19.0	7
Seville	Spain	0	20.0	10

Kinetic modelling

The software package KinGUI version 2 was used for parameter fitting [*Schäfer et al. (2007) – 2007/1062781*]. The error tolerance and the number of iterations of the optimisation 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): "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 2.0, 434 pp., chapter 6.1.4 and chapter 8.3.1*], both spatially and temporally. The limit of quantification (LOQ) for iprodione reported in the study was 0.005 mg kg^{-1}. A limit of detection (LOD) was not provided in the study report and was therefore defined as LOQ. According to FOCUS, values below LOD were set to 0.5 × LOD = 0.0025 mg kg^{-1}.
- Soil residues were reported in mg per kg wet soil and therefore corrected to values in mg per kg dry soil, using the soil moisture content given in the report.
- The measured levels of iprodione at time zero were corrected by adding the measured levels of the metabolite RP 30228.
- 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 the default soil density of 1.5 g cm^{-3}. 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 evaluation was only performed for the parent substance iprodione.

Kinetic models included in the evaluations

The set of models which can be employed for kinetic evaluations was described in the report of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]: Single-First-Order (SFO), Double first-order in parallel (DFOP), First-order multi-compartment (FOMC) and the Hockey-stick (HS) model. The selection was modified by the EFSA opinion [*EFSA (2010)*], excluding the FOMC from the calculation of the $DegT_{50}$ for normalised 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)*].

The appropriate kinetic model was selected in a step-wise approach proposed by the EFSA opinion [*EFSA (2010)*] to derive the appropriate endpoints from each field dissipation trial, and corresponding $DegT_{50}$ values are reported as modelling endpoints.

II. RESULTS AND DISCUSSION

Kinetic evaluation of the original dataset showed that the SFO model is appropriate to derive normalised modelling endpoints for iprodione from the experimental data obtained in all field trials. The rate constants (*k*) and field half-lives ($DegT_{50}$) adequate to be used in environmental fate modelling are summarised in Table 7.1.2.2.1-9.

Table 7.1.2.2.1-9: Summary of endpoints for use in modelling of iprodione

Field trial	Soil type (USDA)	Kinetic model	All data included		Data > 10 mm rainfall only	
			Rate k [d^{-1}]	DT_{50} [d]	Rate k [d^{-1}]	$DegT_{50}$ [d]
Goch	Silt loam	SFO	0.0935	7.4	0.1983	3.5
Lyon	Sandy loam	SFO	0.0661	10.5	0.1501	4.6
Manningtree	Sandy loam	SFO	0.2346	3.0	0.2001	3.5
Seville	Sandy loam	SFO	0.0601	11.5	0.0601	11.5
Geometric mean			**0.0966**	**7.2**	**0.1375**	**5.0**

III. CONCLUSION

Kinetic evaluation of four field trials with iprodione was conducted in order to derive modelling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics and the EFSA scientific opinion on estimating $DegT_{50}$ values in soil for modelling purposes. The field trials were situated in different regions of Europe (Germany, France, UK and Spain), considering a range of different soils and climatic conditions.

Kinetic evaluation of the time-step normalised dataset (20°C, pF2) resulted in normalised field half-lives ($DegT_{50}$) for iprodione between 3.5 and 11.5 days.

Report:	CA 7.1.2.2.1/3 Richter T.,Kuhnke G., 2012a Field soil dissipation study of BAS 610 F (Iprodione) in the formulation BAS 610 06 F on bare soil at two different locations in France, 2010 2011/1284995
Guidelines:	SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995), SANCO/825/00 rev. 7 (17 March 2004), SANCO/3029/99 rev. 4 (11 July 2000), EEC 95/36 of 14 July 1995 amending 91/414/EEC, BBA VI 4-1 (December 1986), ECPA Guidance Document on Field Soil Dissipation Studies Aug. 1997
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The dissipation of iprodione and its metabolite RP 30228 under field conditions was investigated at two sites representing typical regions of agricultural practice in northern and southern France.

Iprodione, formulated as a WG formulation, was broadcast applied to bare soil in a single application at a nominal rate of 1000 g a.s./ha. Following application and day 0 sampling, the substance was incorporated mechanically into the top 10 cm soil horizon.

Soil specimens were taken at intervals up to nominal 200 days and down to a soil depth of 50 cm. Following extraction with acetonitrile/water (70/30, v/v), the soil samples were analysed for iprodione and RP 30228 by HPLC-MS/MS, with an LOQ for iprodione of 0.005 mg/kg and for RP 30228 of 0.004 mg/kg.

In the unfortified control samples the concentrations of iprodione and RP 30228 were below the LOD (< 0.001 mg/kg for iprodione and < 0.0004 mg/kg for RP 30228) in all cases, indicating the absence of interferences. Procedural recovery experiments performed with spiked field soils yielded overall mean recovery rates between 80.4% and 99.8% (iprodione) and between 81.6 % and 98.7 % (RP 30228). Thus the recovery experiments confirmed the validity of the analytical method used in the study.

Iprodione degraded in acidic soils under field conditions at two European sites. At the trial site L100593, about 90 % of the applied amount was degraded after about 200 DAT. The DT_{50} value for this trial is moderate and the calculations are the subject of a separate modelling report. Since the concentration of iprodione of trial L100594 dropped by about 50 % between 0 and 5 DAT due to a thunderstorm with hail and heavy rainfalls after application, these results were not taken for degradation rate calculations. However from 5 DAT on, residues decreased until the end of the study with at least an equal rate as in trial L100593.

Residues of iprodione and its metabolite were generally found only in the upper 10 cm of the soil. No residues above the LOQ were found below this layer, indicating no potential of residues of both compounds to appear in groundwater.

I. MATERIAL AND METHODS

1. Test Material

Test item (formulation): BAS 610 06 F
Active ingredient: Iprodione (Reg.No. 101169)
Type of formulation: WG

2. Test sites

The dissipation of iprodione and its metabolite RP 30228 under field conditions was investigated at two sites in France representative of Northern and Southern EU conditions. The trial L100593 was performed in the west of France (northern area) in the department of Maine-et-Loire. The trial L100594 was performed in the south-west of France (southern area) in the department of Lot.

The site characteristics including the basic soil parameters of the corresponding soil horizons are presented in Table 7.1.2.2.1-10.

Table 7.1.2.2.1-10: Characteristics of the trial sites used to investigate the field dissipation of iprodione and its metabolite RP 30228

Trial	L100593		L100594				
Location	Luigné, France		Saint Denis Catus, France				
Soil properties	0 - 50 cm	50 - 90 cm	0 - 30 cm	30 – 40 cm	40 - 50 cm	50 - 60 cm	60 - 90 cm
Soil texture (DIN 4220)	Medium silty sand	Medium loamy sand	Medium silty sand	High loamy sand	Medium loamy sand	Medium sandy loam	Sandy loamy silt
Sand [%]	60.5	74.4	57.1	52.0	55.7	47.0	40.5
Silt [%]	34.4	15.3	35.6	31.6	33.5	34.5	50.9
Clay [%]	5.0	10.4	7.3	16.3	10.7	18.5	8.6
Soil texture (USDA)	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Loam	Silt loam
Sand [%]	62.6	75.6	60.2	58.7	54.5	50.0	38.9
Silt [%]	30.4	15.7	32.3	30.2	29.0	31.3	51.8
Clay [%]	7.0	8.8	7.4	11.1	16.5	18.6	9.4
Organic C [%]	0.84	0.43	1.17	0.66	0.32	0.21	0.29
Organic matter [%] *	1.45	0.74	2.02	1.14	0.55	0.36	0.50
pH [H_2O]	5.1	5.0	4.8	4.9	4.9	4.8	5.0
pH [$CaCl_2$]	4.3	4.1	4.1	4.1	4.1	4.1	4.3
CEC [mval Ba/100g dry weight]	6.2	6.4	6.7	6.0	6.0	6.2	11.7
MWHC [g/100g dry weight]	37.3	31.9	49.5	45.5	40.2	42.9	39.7

* organic matter = organic carbon x 1.724
CEC = cation exchange capacity
MWHC = maximum water holding capacity

The selected fields represented typical regions of agricultural practice and had been under cultivation for many years. 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 iprodione had been used on the test plots in the last three years.

Each trial area was divided into two plots. One plot (60 m²) was used as control plot (untreated) and the second plot was treated with the test item. Treated plots consisted of four subplots (A, B, C and D) that were assigned for replicates. The size of each treated replicate was 43.2 m^2 (L100593) and 52.8 m² (L100594), respectively.

The control plot was subdivided into two sub-subplots of equal size. Each of the four treated subplots was subdivided into eight sub-subplots of equal size (4.8 m^2 respectively 6 m^2 for each sub-subplot) and two buffer stripes (1 m width each). The width of the treated subplots was 2 or 2.5 m and adapted to the size of the spraying boom used. The buffer strips at the beginning and at the end of each treated subplot were treated with the test item but were not sampled.

The distance between the treated subplots was at least 2 m, the distance between treated and untreated plot was at least 10 m.

3. Experimental treatments

The product, formulated as a WG, was broadcast applied to bare soil in a single application at a nominal rate of 1000 g a.s./ha. The applications were conducted at the end of May 2010 using a calibrated boom sprayer. For each treated replicate, a separate spray mixture was prepared and the test item was applied to each one separately. The actual application rates determined by quantifying the amount of spray discharged ranged from 968 to 1029 g a.s./ha. In addition, the dose was verified by means of sampling Petri dishes (10.8 cm inner diameter) filled with approximately 50 g of standard soil LUFA 2.2, which were placed on the treated plot before application. Details of application are presented in Table 7.1.2.2.1-11.

Table 7.1.2.2.1-11: Application parameters of field trial sites treated with iprodione

Trial Country	Test item/ Nominal content/ Formulation type	Application method	No. of appli-cations	Application rate per treatment			No. of treated replicates	Application date
				Nominal [g a.s./ha]	Actual* [g a.s./ha]	Dose verifi-cation*		
L100593 Northern France	BAS 610 06 F 750 g a.s./kg WG	Broadcast spray to bare soil	1	1000	1012	101% of nominal rate	4 (43.2 m^2 each)	28-May-2010
L100594 Southern France	BAS 610 06 F 750 g a.s./kg WG	Broadcast spray to bare soil	1	1000	1009	101% of nominal rate	4 (52.8 m^2 each)	26-May-2010

* determined by calculation of spray liquid applied; mean of four replicates

The site at Luigné (test site L100593) was not irrigated. At the trial site L100594, irrigation was performed twice during the field phase: at 79 DAT (5.1 mm) and at 97 DAT (8.6 mm). According to the study plan and evapotranspiration data (ETo), 31.9 mm should have been irrigated on the test plot between 0 DAT and 111 DAT but only 13.7 mm were brought in August. There was no need to irrigate until 55 DAT due to the heavy rains recorded between 13 to 24 DAT (227.4 mm). During the period 56 DAT to 97 DAT, the trial missed only 2.4 mm of irrigation that was not applied later. At 111 DAT, the trial received 5.4 mm too much. Afterward, the ETo values did not show the necessity to irrigate the trial until the last sampling.

No tillage or fertilisation 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 glufosinate-ammonium.

Climatic conditions were based on records of appropriate weather stations located on-site or at a distance of maximum 20 km from site. Monthly summary results on temperature and precipitation are presented in Table 7.1.2.2.1-12.

Table 7.1.2.2.1-12: Summary of monthly air temperature and precipitation at each field trial site

Trial	L100593		L100594	
Location	Luigné		Saint Denis Catus	
Country	France		France	
Climatic conditions / Month	T_{mean} Air [°C]	Precipitation [mm]	T_{mean} Air [°C]	Precipitation [mm]
May 10	15.4	8.4	15.3	33.7
Jun 10	18.0	46.9	17.6	230.2
Jul 10	21.0	13.6	21.3	54.7
Aug 10	18.7	31.2	19.5	37.5
Sep 10	15.5	75.9	16.1	116.7
Oct 10	11.5	64.4	11.6	87.2
Nov 10	7.1	68.3	7.0	122.2
Dec 10	0.2	52.7	3.2	10.9

weather data refer to time period from start of trial (day of application) until end of trial (day of last sampling)

4. Sampling

Replicate soil specimens (20 per treated plot and 20 per control plot) were taken at intervals up to about 200 days and down to a soil depth of up to 50 cm. Immediately after application (0 DAT), the treated plots were sampled down to 30 cm depth 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 treatment]
L100593	France, north	-1, 0, 14, 34, 60, 96, 196
L100594	France, south	-1, 0*, 5, 19, 30, 64, 100, 202

*10-30 cm double samples not taken due to bad weather conditions

Untreated specimens were collected from the control plot on two occasions, one day before the application and on the final day of sampling. The 20 specimens were taken randomly from half of the untreated plot each time and pooled according to soil depth.

Treated soil specimens were taken randomly from 5 points of each of the four treated subplots A – D and pooled together according to depth. Immediately after sampling and before freezing, all soil cores collected with the soil probe were sectioned into segments of 10 cm and pooled by depth.

All soil specimens were placed into freezer storage at about -18°C within 6 hours of being taken (with exception of sampling S5 in trial L100593) and remained frozen at about -18°C until processing or analysis of the samples.

5. Description of analytical procedure

The soil and Petri dish specimens were analysed for iprodione and RP 30228 using a modified method based on a validated BASF method. In brief, a 10 g soil sample was extracted twice with 50 mL of acetonitrile/water (70/30, v/v). The combined extracts were filled up to 100 mL with acetonitrile/water (70/30, v/v) and a 10 mL aliquot thereof was added to 40 mL of water and partitioned three times with 10 mL of dichloromethane. The combined organic phases were reduced to dryness under a stream of nitrogen at 40°C. The residues were re-dissolved in 3 mL of acetonitrile/water (70/30, v/v) and analysed by liquid chromatography (LC-MS/MS). Analysis of soil specimens originating from the treated plots was conducted down to a depth ensuring that at least two soil segments were free of iprodione and RP 30228 residues. The method has an LOQ for iprodione of 0.005 mg/kg and for RP 30228 of 0.004 mg/kg.

II. RESULTS AND DISCUSSION

A. METHOD VALIDATION

The average recoveries yielded from fortification experiments were 89.7 ± 5.6%, 80.4 ± 8.7% and 99.8 ± 3.8% for the three fortification levels 0.005, 0.024 and 0.368 mg/kg, respectively. Recovery values for RP 30228 (mean ± RSD) were 81.6 ± 12.3%, 94.7 ± 26.0% and 98.7 ± 3.6% for fortification levels of 0.004, 0.022 and 0.332 mg/kg, respectively.

These data proved that the analytical method applied was able to accurately determine iprodione and RP 30228 residues in soil samples of the sites down to a concentration of 0.004 mg/kg.

In all cases the concentration of iprodione and RP 30228 in the unfortified control samples was below the LOD (< 0.001 mg/kg for iprodione and < 0.0004 mg/kg for RP 30228) indicating the absence of interferences.

B. APPLICATION VERIFICATION

The application rates determined by analysis of the application monitors (Petri dishes filled with soil) represented only about 10% of the target application rate. Considering the application solution actually sprayed as well as the application rates determined by quantifying the amount of spray discharged (968 to 1029 g a.s./ha) and the 0 DAT soil samples (1128 and 1028 g a.s./ha), this is unlikely and had to be the result of a mistake during analysis.

C. FINDINGS

Field soil samples taken from different depths were analysed to a maximum of about 200 days after treatment. Depth increments were analysed at each sampling interval until, as a minimum, two residue-free layers were reached. The analytical results are summarised in Table 7.1.2.2.1-14 and Table 7.1.2.2.1-15. All residue values presented in these tables are related to the dry weight of the soil and were not corrected for procedural recoveries.

Table 7.1.2.2.1-14: Summary of iprodione and RP 30228 residues in treated soil samples of trial L100593 (Luigné, France)

	Iprodione			RP 30228		
Depth [cm]	**0-10**	**10-20**	**20-30**	**0-10**	**10-20**	**20-30**
DAT	**[µg/kg]**	**[µg/kg]**	**[µg/kg]**	**[µg/kg]**	**[µg/kg]**	**[µg/kg]**
0	883	<LOQ	<LOQ	38	<LOQ	<LOQ
14	880	<LOQ	<LOQ	56	<LOQ	<LOQ
34	511	<LOQ	<LOQ	22	<LOQ	<LOQ
60	434	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
96	278	<LOQ	<LOQ	16	<LOQ	<LOQ
196	97	<LOQ	<LOQ	10	<LOQ	<LOQ

DAT = days after treatment

Table 7.1.2.2.1-15: Summary of iprodione and RP 30228 residues in treated soil samples of trial L100594 (Saint Denis Catus, France)

	Iprodione			RP 30228		
Depth [cm]	**0-10**	**10-20**	**20-30**	**0-10**	**10-20**	**20-30**
DAT	**[µg/kg]**	**[µg/kg]**	**[µg/kg]**	**[µg/kg]**	**[µg/kg]**	**[µg/kg]**
0	915	<LOQ*	<LOQ*	12	<LOQ*	<LOQ*
5	455	<LOQ	<LOQ	7	<LOQ	<LOQ
19	227	<LOQ	<LOQ	9	<LOQ	<LOQ
30	230	<LOQ	<LOQ	6	<LOQ	<LOQ
64	162	121	<LOQ	7	<LOQ	<LOQ
100	88	<LOQ	<LOQ	5	<LOQ	<LOQ
202	76	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

DAT = days after treatment
* samples of 10-30 cm were taken only from two replicates (C and D), then thunderstorm occurred

Iprodione was degraded significantly in trial L110593. The analytical data showed that about 90 % of the applied amount was degraded by the end of the study (after about 200 DAT).

Since the concentration of iprodione of trial L100594 dropped by about 50 % between 0 and 5 DAT due to the thunderstorm with hail and heavy rainfalls after application, these results were not taken for degradation rate calculations. However from 5 DAT on, residues decreased until the end of the study with at least an equal rate as in trial L100593.

Quantifiable levels (> 0.005 mg/kg) of iprodione were only found to a depth of 10 cm (with one exception in trial L100594). Metabolite RP 30228 was only present at small concentrations (0.005 – 0.056 mg/kg) up to a depth of 10 cm.

From these results it appears that iprodione has no potential to appear in groundwater.

A detailed kinetic evaluation of the degradation behaviour of iprodione in trial L100593 is presented in a separate modelling report (see CA 7.1.2.2.1/4 for trigger endpoints, CA 7.1.2.2.1/5 for modelling endpoints).

III. CONCLUSION

Iprodione and RP 30228 could be detected in low amounts in soil applying a sensitive LC-MS/MS method with an LOQ 0.005 mg/kg for iprodione and 0.004 mg/kg for RP 30228.

The applied analytical method proved to be applicable to correctly determine residues of iprodione and RP 30228 in soil. This was demonstrated by the absence of interferences in untreated soil as well as excellent procedural recovery rates in field soil.

Iprodione degraded in acidic soils under field conditions at two European sites. At one trial site about 90 % of the applied amount was degraded after about 200 DAT. The DT_{50} value for this trial is moderate and the calculations are the subject of a separate modelling report. Considering the second trial it was obvious that degradation occurred, especially evaluating residues beginning from first sampling after hailstorm, which was at 5 DAT.

Residues of iprodione and its metabolite were generally found only in the upper 10 cm of the soil. No residues above the LOQ were found below this layer, indicating no potential of residues of both compounds to appear in groundwater.

Report: CA 7.1.2.2.1/4
Szegedi K., 2011a
Kinetic evaluation of a BAS 610 F - Iprodione field dissipation study according to recommendations of FOCUS kinetics in order to derive a non-normalised DT50
2011/1285122
Guidelines: SANCO/10058/2005 rev. 2 (FOCUS kinetics report)
GLP: no

Executive Summary

The purpose of the kinetic evaluation was to analyse the degradation kinetics of BAS 610 F – iprodione in acidic soil observed in a field dissipation study situated in France. The kinetic analysis was performed on the non-normalised dataset derived from trial L100593 in order to derive endpoints for use as triggers for additional work according to the guidance of the FOCUS workgroup on degradation kinetics.

The results showed that the degradation of iprodione in the acidic soil followed SFO kinetics with a DT_{50} of 60.3 days and a DT_{90} of 200.2 days.

I. MATERIAL AND METHODS

Kinetic analysis and calculations of DT_{50} and DT_{90} values were performed for the parent compound iprodione following recommendations of the FOCUS Kinetics workgroup [*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, v. 2.0, 434 pp.*] in order to derive endpoints for use as triggers for additional work.

The kinetic evaluation was performed using the non-normalised dataset obtained from a field dissipation study situated in France (trial L100593). A description of the study can be found in CA 7.1.2.2.1/3. Measured (non-normalised) soil residues used for kinetic analysis are presented in Table 7.1.2.2.1-16.

Table 7.1.2.2.1-16: Soil residue data used for modelling

DAT	Total residues* [g/ha]
0	1141
14	1110
34	690
60	619
96	410
196	111

* as initial concentrations of metabolites are expected to be zero the DAT 0 observations were assigned to the parent compound

Kinetic modelling

The kinetic analysis was done by non-linear regression methods using the software package KinGUI version 1.2. The SFO and FOMC kinetic models were employed for fitting according to FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. The appropriate kinetic model was selected under consideration of the "visual fit assessment" and the χ^2 test.

II. RESULTS AND DISCUSSION

The kinetic evaluation showed that SFO represents the best-fit kinetic model. The estimated parameters, statistical indices and the corresponding DT_{50}/DT_{90} values are given in Table 7.1.2.2.1-17.

Table 7.1.2.2.1-17: Estimated parameters and DT_{50} / DT_{90} values for iprodione

Best fit kinetic model	SFO
k [d^{-1}]	0.0115
t-test for k [-]	<0.001*
std dev of k [d^{-1}]	0.0016
M0 [mg ha^{-1}]	1.1785
std dev of M0 [mg ha^{-1}]	0.0631
DT_{50} [d]	60.3
DT_{90} [d]	200.2
χ^2 test [%]	7.64

* parameter significantly different from zero considering a 5% significance level

III. CONCLUSION

The kinetic evaluation showed that SFO was the best-fit model for iprodione, using the non-normalised field data. The estimated DT_{50} was 60.3 days, with a corresponding DT_{90} of 200.2 days.

Report: CA 7.1.2.2.1/5
Platz K., 2011a
Kinetic evaluation of a BAS 610 F - Iprodione field dissipation study according to recommendations of Focus kinetics in order to derive a modelling DT50 endpoint
2011/1252227
Guidelines: FOCUS Kinetics Report SANCO/10058/2005 ver. 2.0
GLP: no

Executive Summary

The degradation kinetics of BAS 610 F – iprodione observed in a field dissipation study situated in France was evaluated according to guidance of the FOCUS workgroup on degradation kinetics.

Prior to kinetic evaluation, data derived from the field studies were normalised to reference conditions by time-step normalisation. Kinetic evaluation was performed on the normalised dataset in order to derive degradation parameters that are valid as modelling endpoints.

The results of the kinetic analysis showed that the SFO kinetic model is appropriate to derive a DT_{50} modelling endpoint for iprodione on basis of the day-length normalised model data obtained in the field trial.

The day-length normalised DT_{50} value of iprodione estimated for the field trial Luigné was 35.3 days.

I. MATERIAL AND METHODS

The kinetic evaluation was conducted for a field dissipation trial with iprodione situated in Luigné (France, trial L100593). A description of the study can be found in CA 7.1.2.2.1/3. Application was in spring to bare soil. Soil characteristics are summarised in Table 7.1.2.2.1-18.

Table 7.1.2.2.1-18: Soil characteristics

Parameter	Trial L100593 0-50 cm	Trial L100593 50-90 cm
Soil type (USDA classification)	Sandy loam	Sandy loam
Particle size distribution [%]		
Sand (50-2000 µm)	62.6	75.6
Silt (2-50 µm)	30.4	15.7
Clay (<2 µm)	7.0	8.7
OC [%]	0.84	0.43
pH ($CaCl_2$)	4.3	4.1

Normalisation procedure

The normalisation procedure was carried out by reducing or increasing day lengths depending on soil temperature and moisture by means of correction factors (f_{temp} and f_{moist}) identical to those used in most regulatory leaching models. Daily soil moisture and soil temperature values were calculated by FOCUS-PEARL 4.4.4 using actual soil characteristics and weather data (temperature, global radiation, precipitation). Based on these model results, daily correction factors for the normalised day length were calculated and the cumulative time between sampling points was determined and used as input for a standard kinetic evaluation according to FOCUS [*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, v. 2.0, 434 pp.*].

The weather data used for the modelling approach (maximum air temperature, minimum air temperature, precipitation, global radiation) were taken from a weather station situated directly at the field trial (11 June to 10 December) and from the nearest meteorological station (27 May - 10 June). The field trial was not irrigated within the study duration.

The actual evaporation of the field trial was estimated within PEARL using the Makkink approach. For the PEARL simulations, two soil profiles were defined on the basis of information from available soil certificates from 0-50 cm and from 50-100 cm. The respective soil properties are given in Table 7.1.2.2.1-18. The lower boundary condition of the simulation profiles was set to 'Free Drainage'.

For description of the hydraulic characteristics, the van Genuchten parameters, which describe the soil-water retention characteristics, were used. The soil hydraulic pedotransfer functions based on the HYPRES database were used.

The bulk of the iprodione residues was situated in the upper 0-10 cm soil layer. Hence, the estimated volumetric water fraction and soil temperature of this layer depth at output depths 1.25 cm, 3.75 cm 6.25 and 8.75 cm were averaged and considered for the soil moisture and soil temperature estimation approach.

Temperature correction factors (f_{temp}) were determined to account for differences between actual daily temperatures as calculated by FOCUS-PEARL 4.4.4 and a reference temperature of 20 °C using the Q_{10} approach according to FOCUS. The Q_{10} response function was applied for temperatures above 0 °C (Equation 7.1.2.2.1-2 b). Below field temperatures of 0 °C it was assumed that no degradation occurs (Equation 7.1.2.2.1-2 b). For the evaluation 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 (θ_{ref}) (Equation 7.1.2.2.1-2 c).

Equation 7.1.2.2.1-2 Calculation of normalised day length based on combination of soil moisture and soil temperature correction factors

a) $D_{norm} = D * f_{temp} * f_{moisture}$

b) $$f_{temp} = \begin{cases} Q_{10}^{\frac{Tact-Tref}{10}} & \text{for } T_{act} > 0°C \\ 0 & \text{for } T_{act} \leq 0°C \end{cases}$$

c) $$f_{moist} = \begin{cases} (\frac{\theta_{act}}{\theta_{ref}})^{B} & \text{for } \theta_{ref} > \theta_{act} \\ 1 & \text{for } \theta_{ref} \leq \theta_{act} \end{cases}$$

with:

D_{norm} =	normalised 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)		[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)	[-]
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 simulated daily volumetric soil moisture values were compared with measured soil moisture provided in the study at the different sampling events in the upper 0 - 10 cm layer. The measured soil moisture values were overestimated by the simulated moistures used for the normalisation approach by trend (conservative approach).

The normalised day lengths were derived according to Equation 7.1.2.2.1-2 a. Table 7.1.2.2.1-19 shows the sampling days for the trial location and the normalised (20°C, pF2) day lengths based on soil moisture and soil temperature data in 0-10 cm depth as simulated by FOCUS-PEARL 4.4.4.

Table 7.1.2.2.1-19: Time-step normalised (temperature and moisture) sampling days

DAT	Day length corrected [d]
0	0
14	7.90
34	18.79
60	36.35
96	58.61
196	102.20

DAT = days after treatment

Kinetic modelling

The software package KinGUI version 1.1 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. In Del Re, A.A.M. et al (Eds): Proceedings of the XIII Symposium of Pesticide Chemistry, Piacenza, 2007, p. 916-923.*]. The error tolerance and the number of iterations of the optimisation tool were set to 0.00001 and 100, respectively.

The iprodione residue dataset was prepared for kinetic evaluation according to FOCUS workgroup [*FOCUS (2006)*]. The reported limit of quantification (LOQ) of iprodione was 0.02 mg kg^{-1}. Measurements below the LOQ were set to the half of LOQ (0.01 mg/kg).

For each sampling point, total residues in the sampled soil core were calculated as sum of residues of the single soil core segments considering the height of the respective segment and the actual soil bulk density.

The datasets derived by time-step normalisation were used as input for SFO kinetic evaluation in order to derive degradation parameters that are valid as modelling endpoints (DT_{50}). According to FOCUS 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.

II. RESULTS AND DISCUSSION

The kinetic evaluation showed that the SFO model is appropriate to derive DT_{50} modelling endpoints for iprodione on basis of the day length normalised model data obtained in the Luigné field trial. The type I error of 0.13 % shows that the degradation rate k is significantly different from zero and the X^2 error that describes the fit quality is low with 8.6 %. The residuals are randomly scattered around zero. The optimised parameters and the statistical results are given in Table 7.1.2.2.1-20.

Table 7.1.2.2.1-20: Optimised parameters and statistics

Parameter	Value	Type I error [%]	X^2 error[%]	Visual assessment
M0 [kg/ha]	1.17	-	8.6	The residuals are randomly scattered around zero
Degradation rate k [1/d]	0.0197	0.13		
DT_{50} [d]	35.3	0.13		

III. CONCLUSION

The kinetic evaluation showed that the SFO model is appropriate to derive a DT_{50} modelling endpoint for iprodione on basis of the day-length normalised data obtained in the Luigné field trial. The estimated day-length normalised DT_{50} value was 35.3 days.

In order to support the validity of the field study from Hardy two storage stability studies were conducted for iprodione and metabolite RP 30228 in sol taken from the sites of the field dissipation study. The results show that iprodione and its metabolite are stable in different soils over 12 to 15 months.

Report: CA 7.1.2.2.1/6
Rosati D. et al., 2001a
Iprodione and its metabolite RP30228: Study 99-165 - Storage stability at about -20°C in soils: Goch (GOC), Sevilla (SEV) and Manningtree (MAN) of the European field soil dissipation No. 16822
C018162
Guidelines: none
GLP: yes

Executive Summary

The objective of the study was to examine the frozen storage stability of iprodione and its metabolite (RP 30228) in soils at three locations in Europe: Manningtree, Seville and Goch. Soil samples (25 g) were fortified at a level of 0.05 mg/kg individually with each compound and at 5 mg/kg with iprodione. Samples were stored in a freezer at -20°C for up to 15 months. At specified storage periods, samples were removed from the freezer and analysed by means of LC-MS/MS. The limit of quantitation (LOQ) was 0.005 mg/kg for all analytes. No significant decline was observed for iprodione and its major metabolite RP 30228. They were stable at least 12 months for the soil from Seville, and at least 15 months for the soils from Manningtree and Goch, under these conditions.

I. MATERIAL AND METHODS

A. MATERIALS

Test materials:	Iprodione	/	Metabolite
	RP 26019		RP 30228
Purity:	99.7%		99.5%
CAS #:	36734-19-7		63637-89-8

B. STUDY DESIGN

1. Experimental Conditions

Soils from three locations, Manningtree (MAN), Seville (SEV) and Goch (GOC) were used in the storage stability study of iprodione and its metabolite (RP 30228). The analytes were dosed to soil samples (25 g) and then stored frozen for 3, 6, 9 (all locations) and 12 (Seville) and 15 months (Goch and Manningtree). 15 samples were prepared with 25 g of soil and fortified with 500 µL of acetonitrile-water (50:50) + 0.1% formic acid. 15 samples were prepared with 25 g of soil and fortified with 500 µL of a solution of iprodione at 250 mg/L in acetonitrile-water (50:50) + 0.1 % formic acid. Further 15 samples were prepared with 25 g of soil and fortified with 500 µL of a solution of iprodione at 2.5 mg/L in acetonitrile-water (50:50) + 0.1% formic acid. Additional 15 samples were prepared with 25 g of soil and fortified with 500 µL of a solution of RP 30228 at 2.5 mg/L in acetonitrile-water (50:50) + 0.1 % formic acid. All samples were stored in a freezer at ≤ -20°C.

Description of analytical procedures

Determination of iprodione and its metabolite (RP 30228)

For determination of iprodione and its metabolite RP 30228, soil samples were analysed by method AR 223-99. The samples were extracted using an Accelerated Solvent Extractor and finally determined by LC-MS/MS. The limit of quantification (LOQ) was 0.005 mg/kg for each analyte. The fortification at 10 x LOQ means 0.050 mg/kg of each product. The fortification at 1000 x LOQ means 5 mg/kg of iprodione.

II. RESULTS AND DISCUSSION

The frozen-storage stability of iprodione and its metabolite RP 30228 in soil over 3, 6, 9 (all locations), 12 (Seville) and 15 months (Goch and Manningtree) of storage was investigated. Soil extracts were examined by LC-MS/MS. The results for iprodione and its metabolite RP 30228 are given in Table 7.1.2.2.1-21.

No significant decline was observed for iprodione and its major metabolite RP 30228. They were stable for up to 12 and 15 months in the dosed stored frozen soil specimens. Complementary results for a French soil can be found in the study C018169 summarised under CA 7.1.2.2.1/7.

Table 7.1.2.2.1-21: Frozen storage stability of iprodione and its metabolite RP 30228 in three soils

Soil	Storage period [months]	Recovery [%]		
		Iprodione (0.5 mg/kg)	Iprodione (5.0 mg/kg)	RP 30228
Goch (GOC)	3	111	92	95
	6	116	98	103
	9	101	82	91
	15	102	95	102
Manningtree (MAN)	3	103	90	95
	6	106	90	101
	9	96	92	90
	15	99	92	101
Seville (SEV)	3	111	64	95
	6	102	103	103
	9	97	90	87
	12	95	90	97

III. CONCLUSION

The resulting data showed that iprodione and its major metabolite RP 30228 remained stable at least 12 months for the soil from Seville, and at least 15 months for the soils from Manningtree and Goch, under these conditions.

Report:	CA 7.1.2.2.1/7 Rosati D. et al., 2001b Iprodione and its metabolite (RP 30228): Study 00-133 - Storage stability at about -20°C in soil: France (FR) of the European field soil dissipation No. 16822 C018169
Guidelines:	none
GLP:	Yes

Executive Summary

The objective of the study was to examine the frozen storage stability of iprodione and its major metabolite RP 30228 in French soil (Pusignan). Soil samples (25 g) were fortified at a level of 0.05 mg/kg individually with each compound and at 5 mg/kg with iprodione. Samples were stored in a freezer at -20°C for up to 9 months. At specified storage periods, samples were removed from the freezer and analysed by means of LC-MS/MS. The limit of quantitation (LOQ) was 0.005 mg/kg for all analytes. No significant decline was observed for iprodione and its major metabolite RP 30228. They were stable for at least 9 months under these conditions.

I. MATERIALS AND METHODS

A. MATERIALS

Test materials:	Iprodione	/	Metabolite
	RP 26019		RP 30228
Purity:	99.7%		99.5%
CAS #:	36734-19-7		63637-89-8

B. STUDY DESIGN

2. Experimental Conditions

Soil from Pusignan (France) was used in the storage stability study of iprodione and its metabolite (RP 30228). The analytes were dosed to soil samples (25 g) and then stored frozen for 3, 6 and 9 months. 15 samples were prepared with 25 g of soil and fortified with 500 µL of acetonitrile-water (50:50) + 0.1% formic acid. 15 samples were prepared with 25 g of soil and fortified with 500 µL of a solution of iprodione at 250 mg/L on acetonitrile-water (50:50) + 0.1% formic acid. Further 15 samples were prepared with 25 g of soil and fortified with 500 µL of a solution of iprodione at 2.5 mg/L in acetonitrile-water (50:50) + 0.1% formic acid. Additional 15 samples were prepared with 25 g of soil and fortified with 500 µL of a solution of RP 30228 at 2.5 mg/L in acetonitrile-water (50:50) + 0.1 % formic acid. All samples were stored in a freezer at ≤ -20 °C.

3. Description of analytical procedures

Determination of iprodione and its metabolite RP 30228

For determination of iprodione and its metabolite RP 30228, soil samples were analysed by method AR 223-99. The samples were extracted using an Accelerated Solvent Extractor and finally determined by LC-MS/MS. The limit of quantification (LOQ) was 0.005 mg/kg for each analyte. The fortification at 10 x LOQ means 0.050 mg/kg of each product. The fortification at 1000 x LOQ means 5 mg/kg of iprodione.

II. RESULTS AND DISCUSSION

The frozen-storage stability of iprodione and its metabolite RP 30228 in soil over 3, 6 and 9 months of storage was investigated. Soil extracts were examined by LC-MS/MS. The results for iprodione and its metabolite RP 30228 are given in Table 7.1.2.2.1-22.

No significant decline was observed for iprodione and its major metabolite RP 30228. They were stable for up to 9 months in the dosed frozen soil specimens. Complementary results for soils from other European sites can be found in in the study C018162 summarised under CA 7.1.2.2.1/6.

Table 7.1.2.2.1-22: Frozen storage stability of iprodione and its metabolite RP 30228 in soil

Soil	Storage period [months]	Recovery [%]		
		Iprodione (0.5 mg/kg)	Iprodione (5.0 mg/kg)	RP 30228
Pusignan (France)	3	96	96	94
	6	100	88	95
	9	93	75	108

III. CONCLUSION

The results obtained from the storage stability study demonstrate that iprodione and its major metabolite RP 30228 remained stable in soil from Pusignan for at least 9 months under these conditions.

Overall summary on Field Dissipation and Degradation Rates

An overview of field dissipation and degradation rates is given in Table 7.1.2.2.1-23.

The following considerations need to be taken into account for the endpoint selection:
Acidic soils:
Studies performed with laboratory soils LUFA 2.2 [*Hartman et al. (2014a) – 2013/1240319, CA 7.1.2.1.1/1*], Sandy loam [*Waring (1993a) – R014527*], and the field site Maine-et-Loire [*Richter and Kuhnke (2012) – 2011/1284995, CA 7.1.2.2.1/3*] provide sufficient information on the behaviour of iprodione in acidic soils. Worst case $DegT_{50}$ determined in soil LUFA 2.2 [*Hartman et al. (2014a) – 2013/1240319, CA 7.1.2.1.1/1; Budde (2014b) – 2013/1611391, CA 7.1.2.1.1/2*] will be used for subsequent calculations.

Alkaline soils:
$DegT_{50}$ determined in field studies will be used for subsequent calculations. $DegT_{50}$ determined in laboratory studies shall be considered as additional information.

Table 7.1.2.2.1-23: Field dissipation studies with iprodione

Field trial	Soil type (USDA)	pH_{CaCl2} [-]	Best-fit model	Trigger-DT_{50} [d]	Modelling-$DegT_{50}$ [d]	Study / Kinetic evaluation
Luigné (France)	Sandy loam	4.3	SFO	60.3	35.3[b]	Richter, Kuhnke (2011/1284995) / Szegedi (2011/1285122) + Platz (2011/1252227)
Saint Denis Catus (France)	Sandy loam	4.1	-[a]	-[a]	-[a]	
Goch (Germany)	Silt loam	6.1	SFO	19.0	3.5[c]	Hardy (2001/1023435) / Budde (2013/1311393)
Lyon (France)	Sandy loam	6.9	SFO	13.5	4.6[c]	
Manningtree (UK)	Sandy loam	6.4	SFO	9.6	3.5[c]	
Seville (Spain)	Sandy loam	7.0	SFO	8.7	11.5[c]	
Geometric mean (soils with a pH>5.0)					**5.0**	

[a] Kinetic evaluation not performed due to heavy rainfall shortly after application and a decline of iprodione concentrations in the soil by about 50% between 0 and 5 DAT. However from 5 DAT on, residues decreased until the end of the study with at least an equal rate as in trial L100593.
[b] DT_{50} according to FOCUS [*FOCUS (2006)*]
[c] $DegT_{50}$ according to EFSA (2010)

CA 7.1.2.2.2 Soil accumulation studies

No soil accumulation studies were performed with iprodione. Field studies show that iprodione is rapidly degraded and multiple applications increase the rate of degradation.

CA 7.1.3 Absorption and desorption in soil

CA 7.1.3.1 Adsorption and desorption

The adsorption/desorption of iprodione was investigated by Burr &Newby [*Burr & Newby (1994) – 1994/1311393*] in four soils and an aquatic sediment. Unfortunately one soil had an organic matter content of only 0.2 % and could therefore no longer be taken into account. A new study on five soil was performed were an attempt was made to determine the sorption of RP 35606 an intermediate compound formed from iprodione at pH values above 6. It is notoriously difficult to synthesize RP 35606. Therefore RP 35606 was generated in situ from iprodione during the experiment and both compounds were measured. In acidic soil the amount of RP 35606 was not high enough to obtain reliable results, but in two alkaline soils the Koc and Freundlich exponent 1/n could be determined. As RP 35606 contains a carboxylic group, so data obtained from the alkaline soils can be considered worst case.

CA 7.1.3.1.1 Adsorption and desorption of the active substance

Report:	CA 7.1.3.1.1/1 Class T.,Walter W., 2014a Determination of the adsorption / desorption behaviour of 14C-BAS 610 F (Iprodione) on 5 soils (OECD Guideline 106) 2013/1311365
Guidelines:	OECD 106 (2000)
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The aim of this study was to determine the adsorption /desorption behaviour of iprodione (^{14}C-BAS 610 F, Reg. No. 101169) on five soils with different chemical and physical properties. Formation of the iprodione intermediate Reg. No. 5079626 (formerly RP 35606) or its degradation product RP 30228 was also observed, with the objective to examine adsorption/desorption behaviour of intermediate RP 35606.

The soils covered a range of pH from 4.1 to 7.4, and a range of organic carbon content from 0.75% to 2.03%. The soils were classified as sandy loam (LUFA 2.2), loamy sand (LUFA 5M), loamy sand (Li 10), sandy clay loam (La Gironda Arahal) and sand (Schifferstadt).

Adsorption kinetics of iprodione were determined at one concentration (2 µg/mL) for two soils, LUFA 5M and Schifferstadt, by equilibration in a soil/solution mixture (1:1) for 2,6,16 and 24 hours. Subsequently, soils where formation of the intermediate was expected were equilibrated with a soil/solution ratio of 1/1 for 16 or 48 hours and the water phases and the (non-acidified) acetonitrile soil extracts were examined by LC/LSC for remaining iprodione and intermediate formed.
Significant adsorption of iprodione at equilibrium was demonstrated for all five soils at a nominal concentration of 2 µg/L.

Adsorption isotherms of iprodione were obtained with all five soils. Equilibrium was established by shaking on a horizontal shaker for 48 hours. The test substance was measured using LC/LSC in the solution phase after equilibrating the soil sample with 0.01 M $CaCl_2$. Soil pellets were extracted once, and the extracts subjected to analysis by LC/LSC.

Freundlich adsorption coefficients (K_F^{ads}) for iprodione ranged from 5.3 to 41.7, resulting in organic carbon normalised values ($K_{F,oc}^{ads}$) from 708 to 2054. Additionally, K_F^{ads} for iprodione intermediate resulted for soils La Gironda (Arahal) of 0.39 and LUFA 5M of 1.4, resulting in $K_{F,oc}^{ads}$ of 32 and 70 for La Gironda (Arahal) and LUFA 5M, respectively. A K_d value of 0.39 mL/g and a K_{oc} value of 46 mL/g were calculated for soil Li 10.

As preliminary tests showed that ^{14}C-iprodione is expected to be not entirely stable in all soil/water systems, no desorption tests were performed prior to soil pellet extraction.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item	^{14}C-BAS 610 F (iprodione)
Reg. No.	101169
Chemical name (IUPAC):	3-(3,5-dichlorophenyl)-*N*-isopropyl-2,4-dioxoimidazolidine-1-carboxamide
Radiochemical Purity:	99.1%
Specific activity:	11.5 MBq/mg (690 dpm/ng)
Molecular weight:	330.2 g/mol

Test item	Intermediate RP 35606
Reg. No.	5079626
Chemical name (IUPAC):	*N*-(3,5-dichlorophenylcarbamoyl)-*N*-isopropylcarbamoyl-glycine
Purity:	about 30% (contains 70% of iprodione)
Molecular weight:	348.2 g/mol

Test item	Isomer RP 30228
Reg. No.	5079647
Chemical name (IUPAC):	*N*-(3,5-dichlorophenyl)-2,4-dioxo-3-isopropylimidazolidine-1-carboxamide
Purity:	99.8%
Molecular weight:	330.2 g/mol

2. Soils

The study was conducted with five different soils originating from Germany. The physico-chemical properties of the soils are provided in Table 7.1.3.1.1-1.

Table 7.1.3.1.1-1: Characterisation of soils used to determine the adsorption / desorption behaviour of Reg. No. 101169 (iprodione)

Soil designation Origin	**LUFA 2.2**	**LUFA 5M**	**Li 10**	**La Gironda (Arahal)**	**Schifferstadt**
Textural class (DIN 4220)	Loamy sand (Sl4)	Loamy sand (Sl2)	Loamy sand (Sl2)	Sandy clay loam	Sand
Soil texture [%], (ISO 11277)					
Sand	55.9	80.0	81.2	48.0	88.1
Silt	32.1	13.9	13.2	24.3	7.7
Clay	12.0	6.1	5.6	27.7	4.3
Textural class (USDA)	Sandy loam	Loamy sand	Loamy sand	Sandy clay loam	Sand
Soil texture [%], (USDA)					
Sand	60.3	82.0	82.8	49.2	88.5
Silt	27.7	11.1	11.6	23.0	7.2
Clay	12.0	6.1	5.6	27.7	4.3
Organic carbon [%] (ISO 10694)	1.47	2.03	0.84	1.22	0.75
Effective CEC [$cmol^+/kg$]	7.6	11.4	5.3	26.3	0.3
pH ($CaCl_2$)	5.4	7.2	6.4	7.4	4.1
pH (H_2O)	5.9	7.9	6.9	8.3	5.0
MWHC [g/100g dry soil]	29.6	25.2	25.1	39.2	27.0
Bulk density [g/L]	1227	1367	1369	1308	1342

B. STUDY DESIGN

1. Experimental conditions

Note:
Under neutral to slightly alkaline conditions iprodione will be converted to the relative stable isomer RP 30228. As an intermediately built compound of this reaction RP 35606 will be present in significant amounts. It is not expected that further degradation occurs under room temperature and neutral conditions. In a water sediment system, the half-life of iprodione was estimated to be < 6 hours, mainly forming RP 35606 (>70%), which dissipated from the water phase forming RP 30228.
One objective of the study was also to examine adsorption / desorption behaviour of intermediate RP 35606. Therefore, LC/LSC was used to separately quantify ^{14}C-iprodione and ^{14}C-intermediate.

Adsorption Kinetics Preliminary Test

Adsorption kinetics testing experiments were performed exemplarily for two soils, loamy sand LUFA 5M (pH 7.2, 2.03% organic carbon) and sand Schifferstadt (pH 4.1, 0.75% organic carbon). Duplicate portions of 2 g dry soil were equilibrated on a horizontal shaker with 2 mL of 0.01 M $CaCl_2$ solution (1:1 soil/solution ratio) in a centrifuge tube overnight. The ^{14}C-iprodione dose solution was added to the soil water to obtain a nominal initial concentration of 2.098 µg in a total volume of 2 mL for LUFA 5M and for Schifferstadt. Test systems were equilibrated on a horizontal shaker for various equilibration times (2, 6, 16 and 24 hours) at room temperature (20°C). After shaking/equilibration and subsequent centrifugation, the supernatant aqueous phase was weighed to obtain the volume of the supernatant and to calculate the portion/volume of the aqueous phase remaining in the soil pellet. The liquid phases obtained after centrifugation and the extracted soil pellets were diluted. All aqueous phases and extracts were analysed for ^{14}C-compounds by direct LSC and LC/LSC.

Adsorption Testing for Four Soils Using LC/LSC and non-acidic extraction

Four soils (LUFA 5M, LUFA 2.2, Li 10 and La Gironda (Arahal)) were pre-equilibrated with a soil/solution ratio of 1/1 overnight. Then ^{14}C-iprodione was added to reach concentrations of 1737 ng in 2 mL for soil LUFA 5M and 1252 ng in 2 mL for soils LUFA 2.2, Li 10 and La Gironda (Arahal). The mixtures were agitated for 16 h (LUFA 5M) or 48 h (LUFA 2.2, Li 10 and La Gironda (Arahal)). Subsequently, the water phases and the acetonitrile soil extracts (without acid) were examined by LC/LSC for remaining iprodione and the iprodione intermediate (Reg. No. 5079626) formed.

Tier 3: Adsorption Isotherms

The Tier 3 tests showed that ^{14}C-iprodione is expected to be not entirely stable in all soil/water systems. Thus for all five soils, the direct method in conjunction with LC/LSC analysis of the soil water and extracts was used to test Freundlich adsorption isotherms, without any desorption steps performed prior to soil pellet extraction.

The test was performed with all soils using an equilibration time of 48 hours and 1:1 soil/solution ratio. Soil samples (2 g) were equilibrated overnight with < 2.0 mL 0.01 M $CaCl_2$ solution. The test substance was added to the mixture to achieve concentrations in the aqueous volume ranging between about 0.03 to about 3 µg/mL. After equilibration for 48 h soil/water specimens were centrifuged, decanted, and aliquots of the aqueous phases were examined by LC/LSC for all three analytes (iprodione, intermediate and isomer). The soil pellet was extracted using acetonitrile as described below and the extract was used for LC/LSC analysis.

2. Description of analytical procedures

Aqueous phase and solid phases (soil pellets) were analysed separately by means of LC-MS/MS. After equilibration the soil/water systems were centrifuged for 5 min at 4000 rpm to separate the soil and water phases; then the water phase was decanted and centrifuged for 30 min at 15,000 rpm to remove soil particles with a diameter of > 0.2 µm. Aliquots of the supernatant were injected (100 µL, full-loop technique) for LC/LSC analysis or used for direct LSC in preliminary experiments.

Determination of ^{14}C-compounds in the soil involved one extraction of soil pellets (2 g) with 5 mL of acetonitrile (for adsorption kinetics experiments acidified acetonitrile was used). Samples were shaken for 5 minutes on a horizontal shaker and sonicated for 30 seconds in an ultrasonic bath, followed by centrifugation (2 min at 3000 rpm). After centrifugation, the supernatant was decanted into a 10 mL volumetric flask and the final volume of 10 mL was adjusted with acetonitrile. An aliquot of the final extract was diluted by a factor of 2 with water for LC/LSC analysis.

II. RESULTS AND DISCUSSION

A. MASS BALANCE AND RECOVERIES

The adsorption kinetics testing gave acceptable mass balances (≥ 94 % after 24 hours) for the acidic sand Schifferstadt after soil pellet extraction. Results for % adsorption obtained from the indirect and from the direct method were nearly identical; LC/LSC of soil water indicated that no intermediate was formed due to the acidity of the soil. For the more basic soil LUFA 5M acceptable mass balances (≥ 93 % after 24 hours) were obtained after soil pellet extraction. Results for % adsorption obtained from the indirect and from the direct method were nearly identical; LC/LSC of soil water, however, indicated that the intermediate was formed due to the alkalinity of the soil.

The following mean mass balances (considering also the intermediate RP 35606) for the adsorption testing in four soils after 16 or 48 h equilibration were obtained: 76% for soil La Gironda (Arahal), 73% for soil LUFA 5M, 81% for soil Li10 and 90% for soil LUFA 2.2.

For mass balance calculations in the adsorption isotherm testing, additionally the peaks observed for the iprodione intermediate and the isomer (if detected) were considered. Mean mass balances over all concentration levels obtained after 48 hours of equilibration time were 92% (LUFA 5M), 84% (LUFA 2.2), 91% (Li 10), 73% (La Gironda (Arahal)) and 73% (Schifferstadt).

B. FINDINGS

Adsorption Kinetics Preliminary Test

The adsorption kinetics on the exemplified two soils (Schifferstadt and LUFA 5M) revealed that adsorption equilibrium is reached after two hours. The intermediate was present in the soil water of the Tier 1 LUFA 5M experiment, but could not be detected in acidified acetonitrile soil extracts due to conversion back to iprodione.

Adsorption Testing for Four Soils Using LC/LSC and non-acidic extraction

Significant adsorption of iprodione at equilibrium was demonstrated for all five soils at concentrations of nominal 1µg/mL (nominal 0.6 µg/mL for soils LUFA 2.2, Li 10 and La Gironda (Arahal) – for details see above).

Tier 3: Adsorption Isotherms

The adsorption isotherm testing resulted for iprodione in Freundlich adsorption coefficients (K_F^{ads}) for the 5 soils in the range from 5.3 to 41.7, resulting in organic carbon normalised values ($K_{F,oc}^{ads}$) from 708 to 2054. Tier 3 tests additionally resulted for the iprodione intermediate in K_F^{ads} for soils La Gironda (Arahal) of 0.39 and LUFA 5M of 1.4, resulting in $K_{F,oc}^{ads}$ for La Gironda (Arahal) of 32 and LUFA 5M of 70. A summary of the experimental results is provided in Table 7.1.3.1.1-2.

In soils Schifferstadt and LUFA 2.2 no ^{14}C-intermediate was generated and observed due to low soil pH.

Because of the soil pH for Li 10 of 6.4 and the soil water pH of 7.0 in this soil/solution system, it was possible to reliably determine the intermediate in the samples with the highest but not in the samples with the lower analyte concentrations. The reached pH in this soil/solution system seems to be the border for the formation of the intermediate. This leads to a K_d value of 0.39 mL/g and a K_{oc} value of 46 mL/g for this soil, but K_F^{ads} and $K_{F,oc}^{ads}$ values could not be calculated.

Table 7.1.3.1.1-2: Summary of adsorption isotherms tests of iprodione on five soils

Soil Name	Soil Type (USDA)	Org. C [%]	pH ($CaCl_2$)	K_F^{ads}		$K_{F,oc}^{ads}$		1/n		K_d [mL/g] Iprodione	K_d [mL/g] Inter-mediate	K_{OC} [mL/g] Inter-mediate
				Iprodione	Inter-mediate	Iprodione	Inter-mediate	Iprodione	Inter-mediate			
LUFA 2.2	Sandy loam	1.47	5.4 (6.5)*	15.9	na	1081	na	0.91	na	9.700	na	na
LUFA 5M	Loamy sand	2.03	7.2 (7.5)*	41.7	1.4	2056	70	0.70	0.78	8.805	na	na
Li 10	Loamy sand	0.84	6.4 (7.0)*	8.98	na	1069	na	0.96	na	6.145	0.39	46
La Gironda (Arahal)	Sandy clay loam	1.22	7.4 (7.5)*	21.1	0.39	1726	32	0.91	1.06	12.165	na	na
Schifferstadt	Sand	0.75	4.1 (6.5)*	5.31	na	708	na	0.96	na	4.050	na	na

* pH of soil water (not based on $CaCl_2$)
na = not applicable

III. CONCLUSION

Significant adsorption of iprodione at equilibrium was demonstrated (preliminary testing) for all five soils. Formation of the iprodione intermediate Reg. No. 5079626 (formerly RP 35606) was observed in basic and slightly acidic soils and used to examine adsorption of this compound.

Adsorption isotherm testing resulted for iprodione in Freundlich adsorption coefficients (K_F^{ads}) for the 5 soils in the range from 5.3 to 41.7, resulting in organic carbon normalised values ($K_{F,oc}^{ads}$) between 708 and 2054.

Adsorption isotherm testing additionally resulted for the iprodione intermediate Reg. No. 5079626 (formerly RP 35606) in Freundlich adsorption coefficients (K_F^{ads}) for soils La Gironda (Arahal) of 0.39 and LUFA 5M of 1.4, resulting in organic carbon normalised values ($K_{F,oc}$) for La Gironda (Arahal) of 32 and LUFA 5M of 70.

Because of the soil pH for Li 10 of 6.4 and the soil water pH of 7.0 in this soil/solution system, it was possible to reliably determine the intermediate in the samples with the highest but not in the samples with the lower analyte concentrations. The reached pH in this soil/solution system seems to be the border for the formation of the intermediate. This leads to a K_d value of 0.39 mL/g and a K_{oc} value of 46 mL/g for this soil, but K_F and K_{Foc} values could not be calculated.

Report: Burr, C.M. & Newby, S.E. (1994): Iprodione: Adsorption/desorption to and from four soils and an aquatic sediment.
Report Rhône-Poulenc Agriculture P94/014 of June 28, 1994.
BASF DocID 1994/1311393
GLP: Yes

The adsorption of ^{14}C-iprodione to and desorption from four soils and an aquatic sediment was investigated to meet the pesticide registration requirements set out in Subdivision N, Section 163-1, EPA Guidelines (1982) and EC Draft Uniform Principles (1993), in accordance with International codes of Good Laboratory Practice.

Table 7.1.3.1.1-3: Characterisation of test soils

Composition	Sandy loam (USDA)	Loamy sand (USDA)	Clay (USDA)	Sand (USDA)	Loam (USDA)
% Sand (0.1-2 mm)	60.47	80.88	7.15	96.44	36.81
% Silt(2-50 µ)	31.04	10.77	39.73	1.62	43.2
% Clay (< 2 µ)	8.48	8.36	53.12	1.93	20.18
% Organic matter	1.9	0.9	2.1	0.2	14.4
pH (0.01M $CaCl_2$)	5.97	6.13	6.01	7.8	5.9
CEC [meq/100g]	5.5	2.2	24.1	2.0	16.8

Results: The adsorption equilibria were achieved within 2 hours for sandy loam, loamy sand, clay and the aquatic sediment (loam) and 0.5 hours for sand.

The Freundlich adsorption K_f values ranged from 0.06 or 0.20 in sand (0.1% organic carbon) to 43 for the aquatic organic sediment (8.7% organic carbon). Values of 2.16 - 2.45 and 6.52 were obtained for soils loamy sand, sandy loam and loam.

When normalised for organic carbon content (K_{oc}) values of 55 (including outlier value) or 202 (excluding outlier value) were obtained for the sand, indicating high to medium mobility in this soil, while for the other soils and sediment, values were in the range 223 to 543 indicated medium to low mobility. Where it was possible to calculate desorption values (K_{des}) these were similar to the adsorption values indicating reversible adsorption.

Table 7.1.3.1.1-4: Calculated Freundlich adsorption coefficients

Soil (USDA)	Organic carbon [%]	K_f	1/n	K_{oc}
Loam	8.5	43.09	0.908	507
Sandy loam	1.1	2.45	0.905	223
Loamy sand	0.5	2.16	0.858	431
Clay	1.2	6.52	0.891	543
Sand	0.1	0.20*	1.021	202

* excluding 0.5 mg/L value (outlier giving very low correlation co-efficient omission of this value gave correlation of 1.0).

Table 7.1.3.1.1-5: Calculated desorption constants

Soil type (USDA)	Desorption cycle									
	1		2		3		4		5	
	K_{des}	1/n	K_{des}	1/n	K_{des}	1/n	K_{des}	1/n	K_{des}	1/n
Loam	49.78	0.908	53.22	0.909	56.61	0.911	58.40	0.911	59.88	0.909
Sandy loam	2.67	0.917	2.26	0.906	nc	nc	nc	nc	nc	nc
Loamy sand	2.64	0.854	2.24	0.793	nc	nc	nc	nc	nc	nc
Clay	8.10	0.882	8.86	0.873	9.28	0.861	9.84	0.853	10.77	0.862
Sand	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc

nc = not calculated

Overall Summary on Soil Adsorption

An overview on adsorption of iprodione to different soils is given in the following table.

Table 7.1.3.1.1-6: Adsorption of iprodione to different soils

Soil	Soil type (USDA)	OC [%]	pH ($CaCl_2$) [-]	k_f [mL/g]	1/n [-]	$K_{f,oc}$ [mL/g]	Study reference
94/5/2	Loam	8.5	5.9	43.09	0.908	507	Burr & Newby (1994/1001651)
94/6/2	Sandy loam	1.1	6.0	2.45	0.905	223	
94/14/2	Loamy sand	0.5	6.1	2.16	0.858	431	
94/15/2	Clay	1.2	6.0	6.52	0.891	543	
97/17/2	Sand	0.1[a]	7.8	0.20	1.021	202	
LUFA 2.2	Sandy loam	1.47	5.4	15.90	0.905	1081	Class (2013/1311365)
Schifferstadt	Sand	0.75	4.1	5.31	0.958	708	
Li10	Loamy sand	0.84	6.4	8.98	0.960	1069	
La Gironda	Sandy clay loam	1.22	7.4	21.1	0.908	1726	
LUFA 5M	Loamy sand	2.03	7.2	41.7	0.696	2056	
Arithmetic mean (n=9)					**0.888**	**927.1**	
Median (n=9)					**0.905**	**708.0**	

[a] Not considered, as organic carbon content does not fulfill guideline requirements (OECD 106)

CA 7.1.3.1.2 Adsorption and desorption of metabolites, breakdown and reaction products

Report: CA 7.1.3.1.2/1
Class T.,Walter W., 2014a
Determination of the adsorption / desorption behaviour of 14C-BAS 610 F (Iprodione) on 5 soils (OECD Guideline 106)
2013/1311365
Guidelines: OECD 106 (2000)
GLP: yes
(certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

The study was summarised under CA 7.1.3.1.1/1 where all details can be found. A short overview on the results of RP 35606 is given in the following executive summary.

Executive Summary

The aim of this study was to determine the adsorption /desorption behaviour of iprodione (^{14}C-BAS 610 F, Reg. No. 101169) and RP 35606 on five soils with different chemical and physical properties. Formation of the iprodione intermediate Reg. No. 5079626 (formerly RP 35606) was also observed, with the objective to examine adsorption/desorption behaviour of intermediate RP 35606.

The soils covered a range of pH from 4.1 to 7.4, and a range of organic carbon content from 0.75% to 2.03%. The soils were classified as sandy loam (LUFA 2.2), loamy sand (LUFA 5M), loamy sand (Li 10), sandy clay loam (La Gironda Arahal) and sand (Schifferstadt)

Adsorption kinetics of iprodione were determined at one concentration (2 µg/mL) for two soils, LUFA 5M and Schifferstadt, by equilibration in a soil/solution mixture (1:1) for 2,6,16 and 24 hours. Subsequently, soils where formation of the intermediate was expected were equilibrated with a soil/solution ratio of 1/1 for 16 or 48 hours and the water phases and the (non-acidified) acetonitrile soil extracts were examined by LC/LSC for remaining iprodione and intermediate formed.

Freundlich adsorption coefficients K_F^{ads} for RP 35606 were 0.39 and 1.4 for soils La Gironda (Arahal) and LUFA 5M respectively, resulting in $K_{F,oc}^{ads}$ of 32 and 70 for La Gironda (Arahal) and LUFA 5M. A K_d value of 0.39 mL/g and a K_{oc} value of 46 mL/g were calculated for soil Li 10.

Report:	CA 7.1.3.1.2/2 Penning H., 2010a Study of the adsorption/desorption behaviour of RP 30228 (metabolite of Iprodione - BAS 610 F) on different soils 2009/1099053
Guidelines:	OECD 106 (2000), EPA 835.1230
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The adsorption and desorption behaviour of non-radiolabelled RP 30228 (metabolite of Iprodione) was investigated in five different soils at three concentrations. The soils covered a range of pH from 5.2 to 7.5, a range of organic carbon content from 0.52% to 3.84% and five different USDA textural classes.

For the determination of the adsorption isotherm, three different nominal concentrations (10, 20, and 30 µg/L) of the test item in 0.01 M $CaCl_2$ solution were used. The ratio of soil versus test solution was 1/25, and the measurements were performed at the adsorption equilibrium time of 48 hours for the five soils. Since RP 30228 was not stable in La Gironda soil, desorption behaviour was not tested for La Gironda soil, but for the four other soils. The amount of RP 30228, which remained adsorbed to the soil from the previous adsorption test, was desorbed in two steps by adding 0.01 M $CaCl_2$ solution without test item.

The following adsorption and desorption parameters were measured and evaluated for RP 30228 in each soil: distribution coefficients K_d and K_{OC} at three concentration levels, Freundlich adsorption coefficients K_F, the Freundlich exponent 1/n, and the corresponding K_{FOC} values.

The K_d values refering to the medium concentration level of 20 µg/L ranged from 24.17 to 171.29 mg/L for the five soils and the corresponding K_{oc} values from 4648 to 10509 mg/L. The Freundlich adsorption coefficients K_F covered a range from 14.16 mL/g to 123.48 mL/g corresponding to K_{FOC} values ranging from 1864 mL/g to 7575 mL/g. Freundlich exponents (1/n) ranged from 0.868 to 0.951.

Desorption was performed in two steps.The values of the constants of desorption $K_{F\text{-}des}$ ranged for Desorption I from 23.66 to 433.74 mL/g and for Desorption II from 45.06 mL/g to 383.27 mL/g withcorresponding values of $K_{FOC\text{-}des}$ ranging for Desorption I from 3810 mL/g to 26610 mL/g and for Desorption II from 5121 mL/g to 35163 mL/g. The Freundlich exponents (1/n) ranged for Desorption I from 0.878 to 1.114 and for Desorption II from 0.880 to 1.108.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item	N-(3, 5-dichlorophenyl)-2, 4-d ioxo-3-isopropylimidazolidine-1-carboxamide (RP 30228)
Reg. No.	5079647
Purity:	99.8 % (study EA2025RF5; study 329753_8 (reanalysis))
Molecular weight:	330.2 g/mol

2. Soils

The study was conducted with five different soils originating from Germany and Spain. The physico-chemical characterisation of the soils is provided in Table 7.1.3.1.2-1.

Table 7.1.3.1.2-1: Characterisation of soils used to investigate the adsorption and desorption behaviour of RP 30228

Soil designation Origin	LUFA 2.1	LUFA 2.3	Nierswalde	Li 10	La Gironda (Arahal)
Textural class (USDA scheme)	Sand	Sandy loam	Silt loam	Loamy sand	Silty clay loam
Soil texture [%], (USDA scheme)					
Sand	89.1	56.8	24.1	81.1	12.7
Silt	8.0	32.0	66.6	13.0	48.3
Clay	2.9	11.2	9.3	6.0	39.0
Organic carbon [%]	0.52	1.09	1.63	0.88	3.84
CEC [$cmol^+/kg$]	2.0	10.4	7.4	5.4	29.0
pH ($CaCl_2$)	5.2	6.9	6.5	5.9	7.5

B. STUDY DESIGN

1. Experimental conditions

In order to determine the adsorption and desorption behaviour of RP 30228 in test soils, air-dried and sieved samples were used. The adsorption / desorption experiments were carried out in duplicate at room temperature of 22±2°C. Due to the low solubility of the test item, the soils were not pre equilibrated with $CaCI_2$ solution, but the $CaCI_2$ solution containing the dissolved test item was added in one step (variation from guideline OECD 106).

Initial experiments, conducted for 24 hours with two soils, revealed that the optimal soil / solution ratio for the adsorption/desorption tests was 1/25 as it has an adsorption of >20% and also allows good quantification of RP 30228 in the solution phase, and was therefore used in the following experiments. The appropriate time for reaching equilibrium conditions was determined to be 48 h from preliminary tests (using LUFA 2.1 and La Gironda soil) and applied to all further experiments.
The adsorption isotherm determination was performed for three concentration levels (nominal concentrations: 10, 20, 30 µg/L), with all five soils. Each experiment (one soil and one solution) was performed in duplicate. The test was performed in 75 mL glass centrifuge vials. 2 g soil was weight in and 50 mL application solution was pipetted to the soil.
The soil/solution suspension was then centrifuged and the mass of the test vessel including contents was determined before and after decanting of the supernatant. An aliquot of the supernatant was sampled and the concentration of test item was determined. RP 30228 in $CaCI_2$ solution and soil was quantified by HPLC-UV. For the soils, in which RP 30228 was stable, the test item adsorbed on the soil was determined based on concentrations in the $CaCl_2$ solution. For La Gironda soil, both phases were analysed.

The amount of test item present in the retained water was taken into account when performing the desorption part for LUFA 2.1, LUFA 2.3, Li 10, and Nierswalde soil. Desorption was carried out in two steps. For desorption step 1, the decanted supernatant from the adsorption experiment was replaced by an equal volume of $CaCl_2$ solution without test item. After shaking this mixture for 48 hours, the test vessels were centrifuged and test item retained in the soil and remaining $CaCI_2$ solution was determined based on gravimetric and chromatographic analysis of the soil after removal of the supernatant. Desorption step 2 was performed in an analogous manner with the soil samples left from desorption step 1.
Subsequent preliminary experiments indicated no interferences with the material used.

2. Description of analytical procedures

RP 30228 in $CaCI_2$ solution and soil was quantified in soil and water by HPLC-UV. For each matrix, two fortification levels were analysed in five replicates. In addition at least one untreated control sample was analysed per matrix and fortification level. 2 g of control soil were extracted for 24 h (250 rpm, 20°C) with 50 mL $CaCl_2$ solution. After centrifugation (3000 rpm, 20°C, 10 min), $CaCI_2$ solution was decanted, and 5 mL of the decanted $CaCl_2$ solution and the remaining soil were used for fortification experiments for either matrix.
For soil, a 2 g soil sample is extracted with 50 mL acetone by mechanical shaking for 60 min at 250 rpm. The extract is centrifuged for 10 min at 3000 rpm (20°C). An aliquot of the extract is evaporated to dryness and reconstituted in water/acetonitrile (75/25, v/v) to the appropriate final volume and measured by HPLC-UV at 250 nm.
For water, 5 mL water is extracted into cyclohexane by mechanical shaking for 60 min at 250 rpm. After centrifugation at 3000 rpm for 2 min, an aliquot of the extract is evaporated to dryness, reconstituted in water/acetonitrile (75/25, v/v) to the appropriate final volume and measured by HPLC-UV at 250 nm.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The adsorption tests gave acceptable mass balances with means from 91.9% to 107.8% (except La Gironda soil with 78.9% indicating irreversible adsorption). This indicates that test item is stable in all soils (except La Gironda). As a result, isotherm determination was done using indirect determination for all soils except La Gironda, where the isotherm adsorption coefficients were determined using the direct method (soil extraction).

B. FINDINGS

With the exception of La Gironda soil, in all soils the stability of the test substance was given. As RP 30228 was stable in LUFA 2.1, LUFA 2.3, Li 10, and Nierswalde soil only the $CaCl_2$ phase was analysed, in the isotherm tests. Due to the instability of RP 30228 in La Gironda soil both phases are analysed in the isotherm test with La Gironda soil.
The Freundlich adsorption coefficients K_F covered a range from 14.16 mL/g to 123.48 mL/g for the five soils. The lowest value was found for LUFA 2.1 soil and the highest value for Nierswalde soil. The K_{FOC} values ranged from 1864 mL/g (La Gironda soil) to 7575 mL/g (Nierswalde soil). Ranging from 1/n = 0.868 to 0.951 the Freundlich adsorption exponent indicated only a slight non-linearity of the adsorption within the tested nominal concentration range from 10 to 30 µg/L.
The values of the constants of desorption K_{Fdes} ranged for Desorption I from 23.66 to 433.74 mL/g and for Desorption II from 45.06 mL/g to 383.27 mL/g. The values of K_{FOCdes} ranged for Desorption I from 3810 mL/g to 26610 mL/g and for Desorption II from 5121 mL/g to 35163 mL/g. Overall, the test item was stable during experiments in four of five soils. These results are summarised in the following tables.

Table 7.1.3.1.2-2: Adsorption of RP 30228 based on Freundlich isotherms in five soils

Soil	Soil Type (USDA)	pH ($CaCl_2$)	K_F [mL/g]	1/n	K_{FOC} [mL/g]	Kd [mL/g][1)]	Koc [mL/g] [1)]
LUFA 2.1	Sand	5.2	14.16	0.884	2723	24.17	4648
LUFA 2.3	Sandy loam	6.9	27.14	0.868	2490	54.04	4958
Li10	Loamy sand	5.9	26.54	0.876	3016	50.21	5706
Nierswalde	Silt loam	6.5	123.48	0.946	7575	171.29	10509
La Gironda	Silt clay loam	7.5	71.59	0.951	1864	91.23	2376

1) The K_d and K_{oc} values given in the table refer to the medium concentration level of the test item (nominal concentration 20 µg/L).

Table 7.1.3.1.2-3: Desorption of RP 30228 based on Freundlich isotherms in four soils

Soil	Soil Type (USDA)	Desorption I (48 h)			Desorption II (66 h)		
		$K_{F\text{-}des}$ [mg/L]	1/n	$K_{FOC\text{-}des}$ [mg/L]	$K_{F\text{-}des}$ [mg/L]	1/n	$K_{FOC\text{-}des}$ [mg/L]
LUFA 2.1	Sand	23.66	0.912	4549	59.00	0.993	11347
LUFA 2.3	Sandy loam	113.45	1.016	10408	383.27	1.108	35163
Li10	Loamy dand	33.52	0.878	3810	45.06	0.880	5121
Nierswalde	Silt loam	433.74	1.114	26610	200.67	0.943	12311

$K_{F\text{-}des}$: Freundlich desorption coefficient
$K_{FOC\text{-}des}$: organic carbon normalised Freundlich desorption coefficient

III. CONCLUSION

The adsorption and desorption behaviour of the test item RP 30228 was determined on five European soils, which covered a range of pH from 5.2 to 7.5, a range of organic carbon content from 0.52% to 3.84%. The soils were classified by USDA scheme into five different textural classes: sandy loam, silt loam, loamy sand, silty clay loam, and one sand.
The Freundlich adsorption coefficients K_F covered a range from 14.16 mL/g to 123.48 mL/g for the five soils. The K_{FOC} values ranged from 1864 mL/g to 7575 mL/g. The values of the constants of desorption K_{Fdes} ranged for Desorption I from 23.66 to 433.74 mL/g and for Desorption II from 45.06 mL/g to 383.27 mL/g. The values of K_{FOCdes} ranged for Desorption I from 3810 mL/g to 26610 mL/g and for Desorption II from 5121 mL/g to 35163 mL/g.

Additional information: The study of Vonk et al. (1993) performed according to Dutch Guidelines from 1991 was no longer considered acceptable due to reported 1/n values ranging from 1.2 to 2.6.

Report: CA 7.1.3.1.2/3
Class T.,Walter W., 2013a
Determination of adsorption and desorption behaviour of Reg.No. 5079618 (metabolite of Iprodione: RP 36221) in 5 soils (OECD guideline 106)
2013/1276030
Guidelines: OECD 106 (2000)
GLP: Yes
(certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The adsorption and desorption behaviour of non-radiolabelled iprodione metabolite (Reg. No. 5079618, RP 36221) was investigated in five different soils. The soils covered a range of pH from 6.2 to 7.4, a range of organic carbon content from 0.99% to 1.63% and different textural classes.
The test substance was determined in the soil aqueous phase (0.01 M $CaCl_2$) and in soil pellets by means of LC-MS/MS. Adsorption kinetics were initially investigated with a 1/10 soil/solution ratio and resulted in very high adsorption which did not allow calculation of accurate results. Subsequently a soil/solution ratio of 1/100 (1 g of soil and 100 mL of aqueous 0.01 M $CaCl_2$ solution) was chosen to study adsorption kinetics for 2, 12, 24 and 48 hours, using the loamy sand LUFA 2.2 (pH 5.5) and the loam Fiorentino Poggio Renatico 1 (PH 7.4). The parallel method was used with determination of the amount adsorbed by the indirect method (decrease of analyte in water phase) as well as via solvent extraction of the soil pellets by the direct method (determination of analyte adsorbed to soil). For the three other soils, adsorption tests with a soil/solution ratio of 1/100 were performed for only 48-hours of equilibration time applying both, indirect and direct method.
Significant adsorption of iprodione metabolite Reg. No. 5079618 (RP 36221) at equilibrium was demonstrated (Tier 1 testing) for all five soils. Tier 1 tests gave acceptable mass balances after 48 hours of equilibration time for the soils LUFA 2.2, and Fiorentino Poggio Renatico 1 in the range from 90 to 96 %, but insufficient mass balance for soils Li 10, LUFA 2.3 and Bruch West in the range from 77 to 87 %. Tier 3 tests resulted in Freundlich adsorption coefficients (K_F^{ads}) for the 5 soils in the range from 133 to 341, resulting in organic carbon normalised values ($K_{F,oc}$) from 13981 to 26591. Desorption coefficients for soil LUFA 2.2 from desorption cycles I and II were significantly higher than adsorption coefficient indicating non-reversible adsorption.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item 1-(3,5-dichlorophenyl)-5-isopropyl biuret
Reg. No. 5079618
Purity: 92.2%
Molecular weight: 290.1 g/mol

2. Soils

The study was conducted with five different soils originating from Germany and Italy. The physico-chemical characterisation of the soils is provided in Table 7.1.3.1.2-4.

Table 7.1.3.1.2-4: Characterisation of soils used to investigate the adsorption and desorption behaviour of Reg. No. 5079618

Soil designation Origin	LUFA 2.2	LUFA 2.3	Bruch West	Li 10	Fiorentino Poggio Renatico 1
Textural class (German scheme, DIN 4220)	Silty sand	Loamy sand	Loamy sand	Silty sand	Loamy sand
Soil texture [%], (German scheme)					
Sand	79.3	66.9	61.1	81.7	41.7
Silt	15.9	24.8	27.6	13,9	41.6
Clay	4.7	8.3	11.3	4.3	16.7
Textural class (USDA scheme)	Loamy sand	Sandy loam	Sandy loam	Loamy sand	Loam
Soil texture [%], (USDA scheme)					
Sand	80.1	68.6	63.7	83.5	49.4
Silt	15.2	23.1	25.1	12.2	33.9
Clay	4.7	8.3	11.3	4.3	16.7
Organic carbon [%]	1.53	0.99	1.63	0.95	1.00
CEC [$cmol^+/kg$]	6.6	7.5	11.9	5.5	11.8
pH ($CaCl_2$)	5.5	6.7	7.3	6.2	7.4
pH (water)	6.2	7.4	8.0	6.9	8.2

B. STUDY DESIGN

1. Experimental conditions

In order to determine the adsorption and desorption behaviour of Reg. No. 5079618 in test soils, air-dried and sieved samples were used.
Solubility of the test substance in water was observed to be rather low. Thus adsorption was assumed to be rather high. Both the indirect and the direct and the parallel method procedures were used. For all adsorption testing experiments, soil was pre-equilibrated with aqueous 0.01 M $CaCl_2$ solution overnight, and then the analyte in aqueous 0.01 M $CaCl_2$ was added to the soil/solution mixture. The mixture was agitated for an appropriate time and then the suspensions were separated by centrifugation. The aqueous phase was analysed (after appropriate dilution) by LC-MS/MS and the amount of analyte adsorbed on the soil was calculated as difference between the amount initially dosed and the amount remaining at the end of the experiment in the aqueous phase (indirect method). Subsequently the soil pellet was extracted to analyse the test item adsorbed to the soil (direct method).

1.1 Adsorption Kinetics Testing for LUFA 2.2 and Fiorentino Poggio Renatico 1

Adsorption kinetics testing experiments were performed for these two soils according to Tier 1. Aliquots of 1.0 g of the air-dried soils were weighed into centrifuge vials and equilibrated with slightly less than 90 mL of 0.01 M $CaCl_2$ aqueous solution over night by shaking. After that, the analyte solution was dosed to the soil water to obtain a nominal initial concentration of 3810 ng in the aqueous phase resulting in a total volume of 100 mL of 0.01 M $CaCl_2$ solution. In total, 8 soil samples were dosed in duplicate per equilibration times up to 48 hours per soil type. In addition, blank soil samples without dosing for blank controls were used. The samples were shaken on a horizontal shaker at 20°C. After equilibration times of 2, 12, 24 and 48 hours, the soil/water systems were analysed for Reg. No. 5079618.

1.2 Adsorption testing for LUFA 2.3, Bruch West, and Li10

For the three soils, the adsorption test was performed with an equilibration time of 48 hours only (Tier 1).

1.3 Tier 3 - Adsorption and desorption isotherms testing

All Tier 3 experiments were performed using a constant soil/solution ratio of 1/100, with 1 g of soil and 100 mL of aqueous 0.01 M $CaCl_2$ solution. Five different analyte concentrations in the range of nominal initial concentrations in the water phase of about 0.4 to about 40 ng/mL were examined.
Adsorption equilibrium was established by shaking dosed soil on a horizontal shaker at 20 °C for about 48 hours. Thereafter the soil/water specimens were centrifuged, decanted, and aliquots of the aqueous phases were diluted for LC/MS/MS determination of the analyte.
For soil LUFA 2.2, adsorption was followed by two 48-hours desorption steps, always with a soil/solution ratio of 1/100. Thus Tier 3 tests resulted for soil LUFA 2.2 in a Freundlich adsorption isotherm by the indirect method and in two consecutive Freundlich desorption isotherms. For the other soils Tier 1 mass balances indicated limited stability thus no desorption was performed, but soil pellet extraction (direct method). These direct method procedures allowed to calculate % adsorption and subsequently K_F and K_{oc} for each of the samples of these four soils separately. Furthermore, Freundlich adsorption isotherms were calculated for the four soils based on results obtained by the direct method.

2. Description of analytical procedures

The test item was determined in the 0.01 M $CaCl_2$ aqueous soil water phase and in soil pellet extracts by methods developed and validated in related studies under PTRL Europe IDs P 2754 G (water) and P 2755 G (soil), using LC-MS/MS. The methods for the determination of the analyte in soil water and in soil pellets were concurrently validated, always resulting in acceptable recoveries and relative standard deviations.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

For soil LUFA 2.2 and Fiorentino Poggio Renatico 1, a complete mass balance was obtained after 48 hours of equilibration time with soil pellet extraction (Tier 1 testing). For the three other soils, adsorption tests with a soil/solution ratio of 1/100 were performed for only 48-hours of equilibration time. Mean mass balances after soil extraction were 81 % for LUFA 2.3, 77 % for Li 10, and 86 % for Bruch West soils (Tier 1 testing), indicating the limited stability of the test item during the experiments.
For the soil LUFA 2.2 Tier 3 testing of adsorption (indirect method) followed by two desorption steps gave a mass balances with a mean of 70 % for adsorption, both desorption steps and at last soil extraction. Tier 3 testing for soil Li 10, Bruch West and LUFA 2.3 was restricted to Freundlich adsorption isotherms testing, with no subsequent desorption steps performed. Soil pellets were extracted for determination of analyte content in soil and mass balance determination which resulted in a mean mass balance of 78 %, 87% and 88 %, respectively. Tier 3 testing for soil Fiorentino Poggio Renatico 1 resulted in a mean mass balance of 90%.

B. FINDINGS

Adsorption kinetics

For soil LUFA 2.2 the percentage of adsorption increased slightly with prolonged equilibration time, reaching both plateaus (direct and indirect) after 48 hours of equilibration time, thus indicating that adsorption equilibrium was reached after 48 hours. For soil Fiorentino Poggio Renatico 1 the percentage of adsorption increased slightly with prolonged equilibration time, reaching a plateau after 48 hours of equilibration time, thus indicating that adsorption equilibrium was reached after 48 hours. For the three other soils, adsorption tests with a soil/solution ratio of 1/100 were performed for only 48-hours of equilibration time.

Adsorption and desorption isotherms testing

All Tier 3 experiments were performed using a constant soil/solution ratio of 1/100, with 1 g of soil and 100 mL of aqueous 0.01 M $CaCl_2$ solution. For soil LUFA 2.2 only, adsorption was followed by two 48-hours desorption steps, always with a soil/solution ratio of 1/100. For the other soils Tier 1 mass balances indicated limited stability thus no desorption was performed, but soil pellet extraction (direct method). Thus Tier 3 tests resulted for soil LUFA 2.2 in Freundlich adsorption isotherms based on the indirect method, and for the other four soils in Freundlich adsorption isotherms based on the direct method. For soil LUFA 2.2 only, two consecutive Freundlich desorption isotherms were obtained by the indirect method.

These results are summarised in the following table.

Table 7.1.3.1.2-5: Adsorption Isotherms Tests of Reg. No. 5079618 in five soils

Soil used			Adsorpt. Indirect Method				Desorption Step I			Desorption Step II		
Soil	Org. C [%]	pH ($CaCl_2$)	K_F^{ads}	$K_{F,oc}^{ads}$	1/n	K_d[1)]	K_F^{des}	$K_{F,oc}^{des}$	1/n	K_F^{des}	$K_{F,oc}^{des}$	1/n
LUFA 2.2	1.53	5.5	341	22303	0.85	322.5	533	34846	0.75	786	51399	0.85
Adsorption Isotherm Results			**Ads. by Direct Method**				Desorption not performed					
Li 10	0.95	6.2	133	13981	0.90	272.0						
LUFA 2.3	0.99	6.7	192	19355	0.86	125.5						
Bruch west	1.63	7.3	280	17153	0.85	262.5						
Fiorentino Poggio Renatico 1	1.00	7.4	266	26591	0.81	110.0						

[1)] The K_d values given in the table refer to the medium value for Tier 1 (48 h equilibration time).

III. CONCLUSION

Significant adsorption of iprodione metabolite Reg. No. 5079618 (RP 36221) at equilibrium was demonstrated (Tier 1 testing) for all five soils. Tier 1 tests gave acceptable mass balances after 48 hours of equilibration time for the soils LUFA 2.2, and Fiorentino Poggio Renatico 1 in the range from 90 to 96 %, but insufficient mass balance for soils Li 10, LUFA 2.3 and Bruch West in the range from 77 to 87 %. Tier 3 tests resulted in Freundlich adsorption coefficients (K_F^{ads}) for the 5 soils in the range from 133 to 341, resulting in organic carbon normalised values ($K_{F,oc}$) from 13981 to 26591. Desorption coefficients for soil LUFA 2.2 from desorption cycles I and II were significantly higher than adsorption coefficient indicating non-reversible adsorption.

Report: CA 7.1.3.1.2/4
Class T.,Walter W., 2013b
Determination of adsorption and desorption behaviour of Reg.No. 207099 (metabolite of Iprodione: RP 25040) in 5 soils (OECD Guideline 106)
2013/1106612
Guidelines: OECD 106 (2000)
GLP: Yes
(certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The adsorption and desorption behaviour of metabolite Reg. No 207099 (formerly RP 25040; metabolite of BAS 610 H - iprodione) was investigated on five different soils. The soils covered a range of pH from 5.5 to 7.4, a range of organic carbon content from 0.95% to 1.63% and different textural classes.
The adsorption equilibrium time was determined for LUFA 2.2 and Fiorentino Poggio Renatico 1 by investigating the adsorption kinetic with direct (analyte adsorbed to soil) and indirect method (decrease of the analyte in water phase) over 2, 12, 24 and 48 hours. Concentrations of metabolite Reg. No 207099 were measured by means of LC-MS/MS. Because the soil mass balance decreased from 96% to 50% in 2 hours in Fiorentino Poggio Renatico 1, indicating limited stability, the calculation by the direct method was corrected for the total remaining analyte. Mean mass balances of further soils, investigated after 24 hours, revealed 90% for LUFA 2.3, 94% for Li 10, and 74% for Bruch West soils.

For the determination of the adsorption isotherm five different concentrations between 5.0 and 500 ng/mL of the test item in 0.01 M $CaCl_2$ solutions were used. The ratio of soil versus test solution was 1/1, and the measurements were performed at the adsorption equilibrium time of 24 hours determined within the study. The following adsorption parameters were measured and evaluated for the test item Reg. No 207099 in each soil: distribution coefficients K_d and K_{OC} at five concentration levels, the Freundlich adsorption coefficient K_F, the Freundlich exponent 1/n, and the corresponding K_{FOC} values. Freundlich adsorption coefficients K_F of 2.45 to 5.15 mL/g were determined corresponding to K_{FOC} values ranging from 230 to 515 mL/g. Freundlich exponents 1/n ranged from 0.87 to 0.90.
Desorption was determined for soil LUFA 2.2 in two steps. The desorption coefficients K_{Fdes1} and K_{Fdes2} accounted for 14.239 to 13.148 mL/g, with corresponding to K_{FOCdes} values of 859 mL/g and 931 mL/g.
Overall, significant adsorption of iprodione metabolite Reg. No. 207099 at equilibrium was demonstrated for all five soils.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item	RP 25040
Reg. No.	207099
Batch No.:	TV2840/F
Purity:	99.6%
Molecular weight:	245.1 g/mol

2. Soils

The study was conducted with five different soils. The physico-chemical characterisation of the soils is provided in Table 7.1.3.1.2-6.

Table 7.1.3.1.2-6: Characterisation of soils used to investigate the adsorption and desorption of iprodione metabolite Reg. No. 207099

Soil designation Origin	LUFA 2.1	Li 10	Lufa 2.3	Bruch West	Fiorentino Poggio Renatico 1
Textural class (German scheme, DIN 4220)	Silty sand	Silty sand	Loamy sand	Loamy sand	Loamy sand
Soil texture [%], (German scheme)					
Sand	79.3	81.7	66.9	61.1	41.7
Silt	15.9	13.9	24.8	27.6	41.6
Clay	4.7	4.3	8.3	11.3	16.7
Textural class (USDA scheme)	Sand	Loamy sand	Sandy loam	Sandy loam	Loam
Soil texture [%], (USDA scheme)					
Sand	80.1	83.5	68.6	63.7	49.4
Silt	15.2	12.2	23.1	25.1	33.9
Clay	4.7	4.3	8.3	11.3	16.7
Organic carbon [%]	1.53	0.95	0.99	1.63	1.00
Organic matter[1] [%]	2.63	1.63	1.70	2.80	1.72
CEC [$cmol^+/kg$]	6.6	5.5	7.5	11.9	11.8
pH ($CaCl_2$)	5.5	6.2	6.7	7.3	7.4
pH (water)	6.2	6.9	7.4	8.0	8.2

[1] organic matter =organic carbon x 1.724

B. STUDY DESIGN

1. Experimental conditions

Adsorption Kinetics Preliminary Test

Adsorption kinetics testing experiments were performed exemplarily for two soils, using the loamy sand LUFA 2.2 (pH 5.5) and the loam Fiorentino Poggio Renatico 1 (pH 7.4). Duplicate portions of 1 g dry soil were equilibrated on a horizontal shaker with slightly less than 1 mL $CaCl_2$ solution (1:1 soil/solution ratio) in a centrifuge tube overnight. Then the analyte dose solution was added to the soil water to obtain a nominal amount of 50 ng/mL in the aqueous phases. Test systems were then equilibrated on a horizontal shaker for various equilibration times (2, 12, 24 and 48 hours) at 20°C. After shaking/equilibration the concentration of the test item was determined in the aqueous phase and in the soil pellet (for indirect and direct determination of adsorption) as described in section 2 below.

Adsorption at one Concentration after 24 h for Additional Soils

For the soils LUFA 2.3, Bruch West and Li 10, adsorption tests with a soil/solution ratio of 1/1 were performed for 24 hours of equilibration time using the same experimental conditions as described above for the adsorption kinetics preliminary test.

Tier 3: Adsorption and Desorption Isotherms

For adsorption determination either the direct or the indirect method can be used. The indirect method involves the analysis of the test item concentration in the aqueous phase and calculation of the difference between the amount initially dosed and the amount remaining in the aqueous phase at the end of the experiment to determine the amount of analyte adsorbed on the soil. The direct method determines the analyte adsorbed by extraction of the soil pellet.
The Tier 1 tests showed that the test item is expected to degrade with extended equilibration times in soils (>24 h), except for the rather acidic soil LUFA 2.2 (pH 5.5). Therefore, for soils LUFA 2.3, Bruch West, Li 10 and Fiorentino Poggio Renatico 1 the direct method for determination of the adsorption isotherm was used and no desorption tests were performed for these soils.
For soil LUFA 2.2 the indirect method was used to determine the adsorption isotherm and the two consecutive desorption isotherms.

All Tier 3 experiments were performed using a constant soil/solution ratio of 1/1, with 1 g of soil and 1 mL of aqueous 0.01 M CaCl solution. Five different analyte concentrations in the range of nominal initial concentrations in the water phase of 5.0 to 500 ng/mL were examined. For all experiments, the soil was pre-equilibrated with aqueous 0.01 M $CaCl_2$ solution, before addition of the test solution. Adsorption equilibrium was established by shaking on a horizontal shaker at 20°C for about 24 hours. Thereafter the soil/water specimens were analysed as described below.

For soil LUFA 2.2, adsorption was followed by two 24-hours desorption steps, by equilibrating the soil pellets twice (desorption steps I and II), each time with fresh 1.0 mL of 0.01 M $CaCl_2$ solution.

Control specimens with only the test item in aqueous 0.01 M $CaCl_2$ solution (adsorption controls) were used to show that no significant adsorption on the surface of the test vessels occurred.

2. Description of analytical procedures

The test item was determined in the 0.01 M $CaCl_2$ aqueous soil water phase and in soil pellet extracts by methods developed and validated in related studies [*Walter & Horton (2013) – 2013/1003162, CA 4.1.2/1; Jooss (2013) – 2013/1003163, CA 4.1.2/3*].

The aqueous phases were centrifuged 30 min at 15,000 rpm to remove soil particles with a diameter of >0.2 mm. Aliquots of supernatants were diluted into acetonitrile/water (1:1, v/v) acidified with 0.1% formic acid before analysis by LC-MS/MS.

Soils pellets (1 g) were extracted three times, first with 2 mL acetonitrile/water (1:1, v/v) acidified with 0.1% formic acid by shaking for 30 min in a horizontal shaker followed by 10 min ultrasonication. After centrifugation (10 min at 4000 rpm) the supernatant was filtered by a glass funnel fitted with glass wool. The extraction was repeated two more times with 2 mL acetonitrile containing 0.1% formic acid. The combined extracts were diluted with acidified acetonitrile/water (1:1 + 0.1%, v/v) before being analysed by LC-MS/MS analysis.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The adsorption kinetics preliminary test and the adsorption testing for one concentration gave acceptable mass balances after 24 h for the rather acidic soils LUFA 2.2 (pH 5.5), LUFA 2.3 (pH 6.7), and Li 10 (pH 6.2) with means from 90% to 102 %, but insufficient mass balances for the more basic soil Fiorentino Poggio Renatico (pH 7.4) with 72 %, and soil Bruch West (pH 7.3) with 74 %, indicating degradation or formation of bound residues. This indicates that the Reg. No. 207099 has a limited stability in more basic soils.

B. FINDINGS

The adsorption kinetics were established on the two exemplarily chosen soils with low and high pH value. The percentage of adsorption of metabolite Reg. No. 207099 slightly increased with prolonged equilibration, with no significant difference between 24 and 48 hours, thus indicating that adsorption equilibrium was reached after 24 hours. Therefore, an equilibration time of 24 h was chosen for further experiments.

Tier 3 tests resulted for soil LUFA 2.2 in a Freundlich adsorption isotherm by the indirect method and in two consecutive Freundlich desorption isotherms.
For all other soils, preliminary mass balances indicated limited stability for longer than 24-hours of analyte/soil contact, thus no additional desorption steps (which would have extended the total equilibration time to 3-times 24 = 72 hours) were considered reasonable. For these four soils, soil pellets remaining after centrifugation/decantation were extracted to obtain the amount of analyte in the soil pellet, with correction for the amount remaining in the residual aqueous phase in the pellet to give the amount of analyte adsorbed.

The adsorption coefficients K_F derived from Freundlich adsorption isotherms for all soils ranged from 2.45 to 5.15 mL/g. This corresponded to K_{FOC} values in the range of 230 to 515 mL/g.
A slight non-linearity of the adsorption with the concentration is evidenced for most of the soils by Freundlich adsorption exponents (1/n) of 0.8 to 0.90. Results are given in Table 7.1.3.1.2-7.

Table 7.1.3.1.2-7: Adsorption parameters of iprodione metabolite Reg. No. 207099 based on Freundlich isotherms in five soils

Soil	Soil type (USDA)	Org. C [%]	pH ($CaCl_2$)	K_F [mL/g]	1/n	K_{FOC} [mL/g]	K_d[1)] [mL/g]
LUFA 2.2	Sand	1.53	5.5	3.516	0.90	230	2.615
Li 10	Loamy sand	0.95	6.2	2.452	0.90	258	2.050
LUFA 2.3	Sandy loam	0.99	6.7	5.057	0.87	511	4.060
Bruch West	Sandy loam	1.63	7.3	4.102	0.89	252	3.395
Fiorentino Poggio Renatico 1	Loam	1.00	7.4	5.151	0.89	515	4.18

[1)] The K_d values refer to the mean value derived at Tier 3 for mean concentrations, corrected for mass balance

The desorption coefficients K_{Fdes} obtained for soil LUFA 2.2 in both desorption steps accounted for 13.148 to 14.239 mL/g. The corresponding K_{FOCdes} values accounted for 859 to 931 mL/g (see Table 7.1.3.1.2-8).
Adsorption was reversible for LUFA 2.2, resulting in higher desorption than adsorption coefficient.

Table 7.1.3.1.2-8: Desorption parameters of iprodione metabolite Reg. No. 207099 based on Freundlich isotherms in soil LUFA 2.2

Soil	Soil Type (USDA)	Org. C [%]	pH ($CaCl_2$)	Desorption 1			Desorption 2		
				K_{Fdes1} [mL/g]	1/n	$K_{FOCdes1}$ [mL/g]	K_{Fdes2} [mL/g]	1/n	$K_{FOCdes2}$ [mL/g]
LUFA 2.2	Sand	1.53	5.5	14.239	1.01	931	13.148	0.97	859

III. CONCLUSION

Significant adsorption of iprodione metabolite Reg. No. 207099, at equilibrium was demonstrated for all five soils. K_{FOC} values ranged from 230 to 515 mL/g.

Desorption isotherms were established for soil LUFA 2.2 demonstrating that adsorption was slightly reversible, resulting in slightly higher desorption than adsorption coefficients. The low mass balances in the rather basic soils Fiorentino Poggio Renatico 1 and Bruch West, indicate that metabolite Reg. No. 207099 has limited stability in more basic soils.

Report: CA 7.1.3.1.2/5
Walter W., 2014a
Determination of adsorption and desorption behaviour of Reg. No. 89517 (Metabolite of Iprodione) in 5 soils (OECD Guideline 106)
2014/1028683
Guidelines: OECD 106 (2000)
GLP: yes
(certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The adsorption behaviour of Reg. No. 89517, metabolite of iprodione, was investigated in five different soils. The soils covered a range of pH from 5.5 to 7.4 and a range of organic carbon content from 0.95% to 1.63%. The soils were classified as sandy loam (LUFA 2.3 and Bruch West), loamy sand (LUFA 2.2 and Li 10) and loam (Fiorentino Poggio Renatico 1).

Adsorption kinetics of Reg. No. 89517 were determined at one concentration (50 ng/mL in the aqueous solution) on two soils, LUFA 2.2 and Bruch West, by equilibration in a soil/solution mixture (1:1) for 2, 4, 6 and 24 hours at 20°C. Adsorption equilibrium was reached after 2 hours. The adsorption isotherms of Reg. No 89517 were established with all five soils, using five concentrations between 5 and 250 ng/mL of the test item in 0.01 M $CaCl_2$ solutions. The ratio of soil versus test solution was 1/1, and measurements were performed at the adsorption equilibrium time of 2 hours. Concentrations of Reg. No. 89517 were measured in both the aqueous phase and in soil extracts by LC-MS/MS.

K_d values ranged from 6.9 mL/g to 15.0 mL/g for an equilibration time of 2 hours. The adsorption coefficients K_F derived from Freundlich adsorption isotherms ranged from 11.981 to 20.251 mL/g with the Freundlich exponent 1/n ranging from 0.80 to 0.85. The organic carbon normalised Freundlich coefficient K_{FOC} values ranged from 1121 to 1655 mL/g.
Desorption was performed in two steps. The desorption coefficients K_{Fdes1} and K_{Fdes2} ranged from 0.609 to 6.798 mL/g for the five soils, with corresponding to K_{FOCdes} values ranging from 45 mL/g to 557 mL/g. Desorption coefficients from desorption cycles I and II were significantly higher than adsorption coefficients.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item	Metabolite of iprodione
Reg. No.	89517
Chemical name (IUPAC):	(3,5-dichlorophenyl)urea
CAS No.:	13142-57-9
Purity:	99.9% (tolerance ± 1.0%)
Molar mass:	205.0 g/mol

2. Soils

The study was conducted with five different soils originating from Germany and Italy. Soil physico-chemical properties are provided in Table 7.1.3.1.2-9.

Table 7.1.3.1.2-9: Characterisation of soils used to determine the adsorption / desorption behaviour of Reg. No. 89517 (iprodione metabolite)

Soil designation Origin	**LUFA 2.2**	**LUFA 2.3**	**Bruch West**	**Li 10**	**Fiorentino Poggio Renatico 1**
Textural class (DIN 4220)	Silty sand	Loamy sand	Loamy sand	Silty sand	Loamy sand
Soil texture [%], (ISO 11277)					
Sand	79.3	66.9	61.1	81.7	41.7
Silt	15.9	24.8	27.6	13.9	41.6
Clay	4.7	8.3	11.3	4.3	16.7
Textural class (USDA)	Loamy sand	Sandy loam	Sandy loam	Loamy sand	Loam
Soil texture [%], (USDA)					
Sand	80.1	68.6	63.7	83.5	49.4
Silt	15.2	23.1	25.1	12.2	33.9
Clay	4.7	8.3	11.3	4.3	16.7
Organic carbon [%] (ISO 10694)	1.53	0.99	1.63	0.95	1.00
Effective CEC [$cmol^+/kg$]	6.6	7.5	11.9	5.5	11.8
pH ($CaCl_2$)	5.5	6.7	7.3	6.2	7.4
pH (H_2O)	6.2	7.4	8.0	6.9	8.2
MWHC [g/100g dry soil]	33.4	28.2	29.2	23.2	29.7
Bulk density [g/L]	1229	1226	1273	1384	1403

B. STUDY DESIGN

1. Experimental conditions

Tier 1: Adsorption Kinetics Preliminary Test

The test was performed with two soils, the loamy sand LUFA 2.2 (pH: 5.5, 1.53% organic carbon), and the sandy loam Bruch West (pH: 7.3, 1.63% organic carbon). Duplicate portions of 2 g dry soil were equilibrated on a horizontal shaker with slightly less than 2 mL $CaCl_2$ solution (1:1 soil/solution ratio) in a centrifuge tube overnight. Then the analyte dose solution was added to the soil water to obtain a nominal amount of 50 ng/mL in the aqueous phase. Test systems were equilibrated on a horizontal shaker for equilibration times of 2, 4, 6, and 24 hours at room temperature (20 – 21°C). After shaking/equilibration and subsequent centrifugation, the supernatant aqueous phase was weighed to obtain the volume of the supernatant and to calculate the portion/volume of the aqueous phase remaining in the soil pellet. The liquid phases obtained after centrifugation were diluted and analysed by LC-MS/MS. After two desorption cycles the soil pellets were extracted and the extracts diluted and analysed by LC-MS/MS.

Adsorption at one Concentration for Additional Soils

For the soils LUFA 2.3, Li 10 and Fiorentino Poggio Renatico 1, adsorption tests with a soil/solution ratio of 1/1 were performed for 2 hours of equilibration time using the same experimental conditions as described above for the adsorption kinetics preliminary test.

Tier 3: Adsorption and Desorption Isotherms

The preliminary tests showed that the test item is expected to degrade with extended equilibration time in soils. The Tier 3 test was performed with all soils using an equilibration time of 2 hours and 1/1 soil/solution ratio. Soil samples (2 g) were equilibrated overnight with < 2.0 mL 0.01 M $CaCl_2$ solution. The test substance was added to the mixture to achieve concentrations of 5, 25, 50, 250 and 500 ng/mL in the aqueous phase (total volume 2 mL). Duplicate samples were used for each concentration and for each soil. The test systems were equilibrated by shaking for 2 hours on a horizontal shaker at 20 – 21°C. After equilibration the aqueous phases were analysed as described in the analytical procedures. Adsorption was followed by two 2 hour desorption steps, always with a soil/solution ratio of 1/1. The two consecutive Freundlich desorption isotherms were obtained by the indirect method.

2. Description of analytical procedures

Aqueous and solid phases (soil pellets) were analysed separately. After equilibration the samples were centrifuged for 5 min at 4000 rpm to separate the soil and water phases. An aliquot of the water phase was centrifuged for 30 min at 15,000 rpm to remove soil particles with a diameter of >0.2 µm. Aliquots of supernatant were diluted volumetrically into acetonitrile / water (1/1, v/v, acidified with 0.1% formic acid) for LC/MS/MS analysis.

Determination of Reg. No. 89517 in the soil involved one extraction of soil pellets with 5 mL of acetonitrile/water (1/1 v/v) + 0.1% formic acid and two extractions with 5 mL of acetonitrile containing 0.1% formic acid. For separation of soil and solvent the samples were centrifuged for 5 minutes at 4000 rpm. Subsequently, the extracts were combined and diluted. An aliquot of the final extract was diluted by a factor of 10 with acetonitrile/water (1/1 v/v) + 0.1% formic acid and subjected to LC-MS/MS analysis.

The soil extraction method was concurrently validated for all five soils at two fortification levels (5.0 and 50 ng/g).

II. RESULTS AND DISCUSSION

A. MASS BALANCE

In the adsorption kinetics testing mass balances of ≥ 95 % were obtained for loamy sand LUFA 2.2 after soil pellet extraction (except for 24 hours equilibration: mass balance ≥ 93 %).
For sandy loam Bruch West mass balances of ≥ 89 % were obtained after soil pellet extraction (except for 24 hours equilibration: mass balance ≥ 77%).

For the adsorption testing of soils LUFA 2.3, Li 10 and Fiorentino Poggio Renatico 1 mass balances were between 90 and 95% considering individual samples.

For the adsorption isotherm the mass balances obtained after 2 hours of equilibration time were 98 – 111 % (5 to 500 ng/mL).

B. FINDINGS

The adsorption kinetics were established on the two exemplarily chosen soils with low and high pH value. The percentage of adsorption of Reg. No. 89517 decreased with prolonged equilibration, reaching a plateau (Adsorption measured directly ≈ 74 % to 93 %) after 2 to 6 hours of equilibration time. Therefore, an equilibration time of 2 h was chosen for further experiments. Adsorption after 2 hours of equilibration (Adsorption measured directly) in additional soils was mostly > 90 %.

K_d values ranged from 6.9 to 15.0 mL/g. The adsorption coefficients K_F derived from Freundlich adsorption isotherms ranged from 11.981 to 20.251 mL/g with the Freundlich exponent 1/n ranging from 0.80 to 0.85. The organic carbon normalised Freundlich coefficient K_{FOC} values ranged from 1121 to 1655 mL/g. A summary of the experimental results is provided in Table 7.1.3.1.2-10.

Table 7.1.3.1.2-10: Adsorption of Reg. No. 89517 based on Freundlich isotherms in five soils

Soil	Soil Type (USDA)	Org. C [%]	pH ($CaCl_2$)	K_F [mL/g]	1/n	K_{FOC} [mL/g]	K_d [1)] [mL/g]
LUFA 2.2	Loamy sand	1.53	5.5	20.251	0.85	1324	15.0
Bruch West	Sandy loam	1.63	7.3	18.280	0.84	1121	12.7
LUFA 2.3	Sandy loam	0.99	6.7	16.389	0.80	1655	12.8
Li 10	Loamy sand	0.95	6.2	11.981	0.83	1261	6.9
Fiorentino Poggio Renatico 1	Loam	1.00	7.4	15.640	0.81	1564	11.9

[1)] The K_d values refer to the mean value derived at Tier 1 (2 h equilibration time)

The desorption coefficients K_{Fdes} obtained in both desorption steps ranged from 14.541 to 46.656 mL/g. The corresponding K_{FOCdes} values were in the range of 1531 to 4713 mL/g (Table 7.1.3.1.2-11).

Table 7.1.3.1.2-11: Desorption of Reg. No. 89517 based on Freundlich isotherms in five soils

Soil	Soil Type (USDA)	Org. C [%]	pH ($CaCl_2$)	Desorption 1			Desorption 2		
				K_{Fdes1} [mL/g]	1/n	$K_{FOCdes1}$ [mL/g]	K_{Fdes2} [mL/g]	1/n	$K_{FOCdes2}$ [mL/g]
LUFA 2.2	Loamy sand	1.53	5.5	41.055	0.84	2683	34.881	0.82	2280
Bruch West	Sandy loam	1.63	7.3	36.739	0.80	2254	31.189	0.78	1913
LUFA 2.3	Sandy loam	0.99	6.7	46.656	0.73	4713	35.573	0.74	3593
Li 10	Loamy sand	0.95	6.2	18.098	0.88	1905	14.541	0.86	1531
Fiorentino Poggio Renatico 1	Loam	1.00	7.4	39.636	0.77	3964	29.108	0.77	2911

III. CONCLUSION

Significant adsorption of Reg. No. 89517, metabolite of iprodione, at equilibrium was demonstrated (Tier 1 testing) for all five soils.
Tier 1 tests gave acceptable mass balances for the earlier stages of the equilibrium and indicate degradation or formation of residues with prolonged equilibration time.
Tier 3 tests gave adsorption coefficients (K_{Fads}) for the 5 soils in the range from 11.981 to 20.251, resulting in organic carbon normalised values (K_{foc}) from 1121 to 1655 mL/g.
Desorption coefficients from desorption cycles I and II were significantly higher than adsorption coefficients.

Report: Feung, C.S. (1999): Iprodione metabolite: ^{14}C-3,5-dichloroaniline soil adsorption/desorption study.
Report Rhône-Poulenc 99R16744 of December 9, 1999.
BASF DocID 1999/1009598 (C022389)
GLP: Yes

The objective of this study was to determine the extent to which 3,5-dichloroaniline is adsorbed to and desorbed from four soils (sandy loam, loamy sand, silt loam and clay soils) and a pond sediment.
The study was conducted in the dark, at a temperature of 25 ± 1°C, at four different concentrations (0.04, 0.12, 0.4 and 0.8 ppm) in a 0.01 M$CaCl_2$ solution (equivalent to 0.2, 0.6, 2 and 4 ppm, respectively, at a soil:water ratio of 1:5).
Physical and chemical properties of the four types of soil and pond sediment are summarised in the following table.

Table 7.1.3.1.2-12: Soil characterisation of soils and pond sediment

Soil or sediment	Soil				Sediment
Collection location	Madera, CA	Clayton, NC	Columbia, MO	Leland, MS	Clayton, NC
Textural classification	**Sandy loam***	**Loamy sand**	**Silt loam***	**Clay**	**Sand**
pH	7.30	6.10	6.80	7.10	6.10
CEC [meq/100g]	4.06	7.63	16.69	37.59	1.65
Organic matter [%]	0.59	2.05	4.49	1.89	1.08
WHC [%] at 1/3 bar	7.00	7.27	27.25	38.02	4.70
WHC [%] at 15 bar	2.07	2.80	12.09	20.72	2.77
Sand [%]	56.40	78.4	10.40	24.40	92.4
Silt [%]	34.00	14.00	66.00	18.00	0.00
Clay [%]	9.60	7.60	23.60	57.60	7.60
Bulk density	1.56	1.52	1.15	1.31	1.42
Heterotrophic plate count [Avg CFU/g]	10100000	12500000	18500000	890000	3150000

* sandy loam and silt loam were used in an aerobic soil metabolism study for 3,5 dichloroaniline.

Results & conclusion: The preliminary range-finding study was conducted with sandy loam and clay and showed that adsorption equilibrium was reached in 13 to 14 hours. No significant degradation was observed by the end of 24 hour adsorption period. No adsorption of 3,5-dichloroaniline to glass containers was observed. The desorption equilibrium was reached at about 13 hours.
Therefore, the adsorption cycle was carried out for 24 hours and each desorption cycles was also carried for 24 hours in the definitive study. Three desorption cycles as well as three organic extractions were carried out.

The presence of the 3,5 dichloroaniline was determined by HPLC and confirmed by LC/MS. Average total radioactivity recovery for all the soils ranged from 84% to 91%.

The average % recovery of applied 3,5-dichloroaniline in the supernatants, organic extracts and soil residues were as follows:

Table 7.1.3.1.2-13: Mass balance of 3,5-dichloroaniline

Soil type	% of applied dose								
	Adsorp.	Desorb.1	Desorb.2	Desorb.3	Extr.1	Extr.2	Extr.3	Solid residue	Total recovery
Sandy loam	55.56	11.96	3.63	1.42	3.54	0.47	1.40	12.88	990.85
Loamy sand	17.21	6.18	3.46	2.04	13.76	1.73	2.91	40.56	87.85
Silt loam	14.01	6.93	4.18	2.54	11.05	1.67	1.75	42.00	84.13
Clay	17.73	9.87	6.07	4.03	21.72	3.40	1.90	21.38	86.10
Pond sediment	28.66	4.50	1.59	0.61	6.03	1.13	3.56	42.12	88.20

The Freundlich adsorption (K_f) values range from 2.029 for sandy loam to 10.221 for clay with an average of 6.907. Adsorption K_{oc} values ranged from 380 for silt loam to 932 for clay with an average of 664. Therefore, 3,5-dichloroaniline would generally be classified as having low mobility according to McCall.

The first desorption constant (K_{des1}) ranged from 4.243 to 19.445. The second desorption constant (K_{des2}) ranged from 9.402 to 23.741 and the third desorption constant (K_{des3}) ranged from 16.489 to 189.452. Reasonably similar K_f and K_{des1} values for the soils and sediment indicated a generally reversible equilibrium between adsorption and the first desorption phases.

Table 7.1.3.1.2-14: Adsorption and desorption coefficients for 3,5-dichloroaniline

Soil type	% Organic matter	Adsorption			Desorption		
		K_r(= K_d)	K_{oc}	1/n	K_{des1}	K_{des2}	K_{des3}
Sandy loam	0.59	2.029	593	0.7075	5.012	9.402	23.372
Loamy sand	2.05	7.446	626	0.6404	19.445	22.909	55.017
Silt loam	4.49	9.906	380	0.6767	15.321	17.211	34.618
Clay	1.89	10.221	932	0.7796	14.358	18.076	16.489
Pond sediment	1.05	4.934	788	0.6578	15.823	23.741	189.452
Average	**-**	**6.907**	**664**	**0.6924**	**13.826**	**-**	**-**

Overall Summary on Soil Adsorption

An overview on adsorption of the metabolites of iprodione to different soils is given in the following table.

Table 7.1.3.1.2-15: Adsorption of the metabolites of iprodione to different soils

Soil	Soil type (USDA)	OC [%]	pH ($CaCl_2$) [-]	k_f [mL/g]	1/n [-]	$K_{f,oc}$ [mL/g]	Study reference
RP 25040							
LUFA 2.2	Loamy sand	1.53	5.5	3.516	0.90	230	Class & Walter (2013/1106612)
Li 10	Loamy sand	0.95	6.2	2.452	0.90	258	
LUFA 2.3	Sandy loam	0.99	6.7	5.057	0.87	511	
Bruch West	Sandy loam	1.63	7.3	4.102	0.89	252	
Fiorentino Poggio Renatico 1	Loam	1.00	7.4	5.151	0.89	515	
Arithmetic mean				***4.056***	***0.89***	***353***	
RP 35606							
LUFA 2.2	Sandy loam	1.47	5.4	n.d.	n.d.	n.d.	Class (2013/1311365)
Schifferstadt	Sand	0.75	4.1	n.d.	n.d.	n.d.	
Li10	Loamy sand	0.84	6.4	n.d.	n.d.	n.d.	
La Gironda	Sandy clay loam	1.22	7.4	0.39	1.063	32.0	
LUFA 5M	Loamy sand	2.03	7.2	1.40	0.781	69.8	
Arithmetic mean					***0.922***	***50.2***	
RP 30228							
LUFA 2.1	Sand	0.52	5.2	14.16	0.884	2723	Penning (2009/1099053)
LUFA 2.3	Sandy loam	1.09	6.9	27.14	0.868	2490	
Li10	Loamy sand	0.88	5.9	26.54	0.876	3016	
Nierswalde	Silt loam	1.63	6.5	123.48	0.946	7575	
La Gironda	Silt clay loam	3.84	7.5	71.59	0.951	1864	
Arithmetic mean				***52.6***	***0.905***	***3534***	
RP 36221							
LUFA 2.2	Loamy sand	1.53	5.5	341	0.85	22303	Class & Walter (2013/1276030)
Li 10	Loamy sand	0.95	6.2	133	0.90	13981	
LUFA 2.3	Sandy loam	0.99	6.7	192	0.86	19355	
Bruch West	Sandy loam	1.63	7.3	280	0.85	17153	
Fiorentino Poggio Renatico 1	Loam	1.00	7.4	266	0.81	26519	
Arithmetic mean				***242***	***0.85***	***19862***	

DCA							
Madera	Sandy loam	0.59	7.3	2.029	0.708	593	Feung (C022389)
Clayton	Loamy sand	2.05	6.1	7.446	0.640	626	
Columbia	Silt loam	4.49	6.8	9.906	0.677	380	
Leland	Clay	1.89	7.1	10.221	0.780	932	
Clayton sediment	Sand	1.08	6.1	4.934	0.658	788	
Arithmetic mean				***6.907***	***0.692***	***664***	
Reg. No. 89517 (LS 720942)							
LUFA 2.2	Loamy sand	1.53	5.5	20.251	0.85	1324	Walter (2014/1028683)
Bruch West	Sandy loam	1.63	7.3	18.280	0.84	1121	
LUFA 2.3	Sandy loam	0.99	6.7	16.389	0.80	1655	
Li 10	Loamy sand	0.95	6.2	11.981	0.83	1261	
Fiorentino Poggio Renatico 1	Loam	1.00	7.4	15.640	0.81	1564	
Arithmetic mean				***16.508***	***0.826***	***1385***	

CA 7.1.3.2 Aged sorption

No studies to investigate aged sorption properties were performed with iprodione or its metabolites.

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

A column leaching study with iprodione was performed by Gouot et al. [Gouout et al. (1976) – C022367. Four columns with different soils were treated with ^{14}C labelled iprodione. The radioactive residues found in the leachate were below 2%. This study is considered obsolete, as sorption and degradation data for iprodione are available and groundwater modelling is a better tool to assess the leaching potential of iprodion.

In an aged leaching study, no leaching was observed in 3 soils while in a sand residues of iprodiones and metabolites were detected.

Report: Newby, S.E. et al. (1994): Iprodione: Aged leaching study in four soils. Report Rhône-Poulenc Agriculture 200638 (P94/013) of July 21, 1994. Report R014529
GLP: Yes

The leaching of ^{14}C-iprodione, after incubation for 30 days, has been studied in four soils to meet the pesticide registration requirements set out in Subdivision N, Section 163-1, EPA Guidelines (1982), in accordance with international codes of Good Laboratory Practice.

Table 7.1.4.1.1-1: Characterisation of test soils

Composition	Sandy loam (USDA)	Loamy sand (USDA)	Clay (USDA)	Sand (USDA)
% Sand (0.1-2 mm)	60.47	80.88	7.15	96.44
% Silt (2-50 µm)	31.04	10.77	39.73	1.62
% Clay (< 2 µm)	8.48	8.36	53.12	1.93
% Organic matter	1.9	0.9	2.1	0.2
pH (0.01M $CaCl_2$)	5.97	6.13	6.01	7.8
CEC [meq/100g]	5.5	2.2	24.1	2.0

^{14}C-iprodione was applied to four soils at a rate equivalent to 10 kg ai./ha and after 30 days incubation, the "aged" soils were transferred to prepared leaching columns to give a nominal depth of 36 cm soil in each column. During this time the soils were maintained under aerobic conditions in the dark at 20°C ± 1°C except on 12 occasions in the month of June when the temperature was between 23°C and 25°C.

The soils were leached over a period of 8 to 10 days with 1040 mL of 0.01M aqueous Calcium chloride, equivalent to 50.8 cm of rainfall. Leaching was carried out in the dark at 20°C ± 1°C except on 12 occasions in the month of June when the temperature was between 23°C and 25°C.

At the end of the leaching period, each column was dismantled and the soil sections extracted. Leachate and soil extracts containing > 1% of the applied radioactivity were analysed by HPLC and the analysis confirmed by liquid chromatography/mass spectrometry and/or by gas chromatography/mass spectrometry.

Results: Acceptable recoveries between 90.7% and 98.5% were achieved for all soils. Less than 1% of the applied radioactivity was produced as volatiles during the incubation and leaching period.

The majority of the applied radioactivity remained in the 0-18 cm segments. Only a small amount (less than 1%) of the applied radioactivity was detected in the leachate from these soils.

Table 7.1.4.1.1-2: Leachate concentrations in different soils

Soil type	% Applied in leachate	Mean K_d	Residue in soil column
Sandy loam	0.35	824	Majority in 0 - 12 cm depth
Loamy sand	0.51	888	Majority in 0 - 18 cm depth
Clay	0.03	10609	Majority in 0 - 12 cm depth
Sand	52.18	41	Majority in 0 - 18 cm depth, but spread throughout column

The major degradates observed were RP 30228 (iprodione isomer), RP 32596 (3,5-dichloroaniline) and "bound residues". Less than 1% of the applied radioactivity was produced as volatiles during the incubation and leaching period.

From sand soil, 52.2% of the applied radioactivity was detected in the leachate: RP 35606 was the major metabolite (27.1%) found in the leachate; iprodione and its isomer RP 30228 accounted for 6.3% and 13.1%, respectively, of the applied radioactivity recovered in the leachate.

Iprodione	6.3% of applied material
RP 25040	2.0%
RP 32596	3.2%
RP 30228	13.2%
RP 35606	27.1%

Table 7.1.4.1.1-3: Composition of radioactivity (% of applied) in sandy loam soil

Sandy loam soil	0 - 6 cm	6 - 12 cm	12 - 18 cm	18 - 24 cm	24 - 30 cm	30 - 36 cm
Iprodione	4.9	5.7	1.5	na	na	na
RP 25040	1.3	nd	0.1	na	na	na
RP 32596	15.0	6.9	0.7	na	na	na
RP 36221	1.0	nd	nd	na	na	na
RP 30228	4.8	nd	nd	na	na	na

nd = not detected
na = not assayed (< 1% of applied radioactivity)

Table 7.1.4.1.1-4: Composition of radioactivity (% of applied) in loamy sand soil

Loamy sand soil	0 - 6 cm	6 - 12 cm	12 - 18 cm	18 - 24 cm	24 - 30 cm	30 - 36 cm
Iprodione	14.4	14.2	10.6	4.9	2.3	na
RP 25040	0.9	0.8	0.4	nd	nd	na
RP 32596	2.9	1.2	0.7	0.3	nd	na
RP 36221	nd	nd	nd	nd	nd	na
RP 30228	12.3	2.8	2.3	0.9	0.5	na

nd = not detected
na = not assayed (< 1% of applied radioactivity)

Table 7.1.4.1.1-5: Composition of radioactivity (% of applied) in clay soil

Clay soil	0 - 6 cm	6 - 12 cm	12 - 18 cm	18 - 24 cm	24 - 30 cm	30 - 36 cm
Iprodione	55.3	17.4	na	na	na	na
RP 25040	1.7	0.5	na	na	na	na
RP 32596	3.2	0.7	na	na	na	na
RP 36221	0.5	nd	na	na	na	na
RP 30228	8.2	1.4	na	na	na	na

nd = not detected
na = not assayed (< 1% of applied radioactivity)

Table 7.1.4.1.1-6: Composition of radioactivity (% of applied) in sand soil

Sand soil	0 - 6 cm	6 - 12 cm	12 - 18 cm	18 - 24 cm	24 - 30 cm	30 - 36 cm
Iprodione	1.8	0.1	nd	nd	0.1	0.1
RP 25040	nd	nd	nd	nd	nd	nd
RP 32596	0.6	0.5	0.8	0.8	1.1	1.1
RP 36221	nd	nd	nd	nd	nd	nd
RP 30228	13.0	4.1	4.1	3.8	4.2	3.9

nd = not detected
na = not assayed (< 1% of applied radioactivity)

The following distribution coefficients (K_d) were obtained by extrapolation (assuming linear leaching): 824 for sandy loam, 888 for loamy sand, 10609 for clay and 4 for sand.
These results indicate that iprodione and its metabolites exhibit low to moderate mobility in three of the soils (sandy loam, loamy sand and clay) but high mobility in sand.

Conclusion: The study shows that in three out of four soils, iprodione and its metabolites show low leaching potential. The exception is the sandy soil. While the metabolites detected in the sandy soil could be expected after incubation in an alkaline soil, the mobility observed in the sandy soil will not be representative for the compounds. The organic matter content of the sand used was only 0.2%, which is far below the recommendation by SETAC (Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides 1995), were an organic matter content of 1.5% to 2.5% is recommended. In the OECD guideline 312 (proposed new guideline 2003) a C_{org} > 0.3 is required (organic metter > 0.5%) lower organic matter contents give no reliable results.

CA 7.1.4.1.2 Column leaching of metabolites, breakdown and reaction products

See chapter 7.1.4.1.1 [*Newby et al. (1994) – R014529*]. The study was conducted with aged soil, so it can be expected that metabolites had already been formed before the leaching phase and so metabolites were also covered by the study.

CA 7.1.4.2 Lysimeter studies

No lysimeter studies were performed with iprodione.

CA 7.1.4.3 Field leaching studies

No field leaching studies were performed with iprodione.

CA 7.2 Fate and behaviour in water and sediment

Data submitted in the previous Annex-I review gave a clear picture of the fate of iprodione in aquatic systems. Iprodione was stable to hydrolysis at pH 5, but was rapidly degraded at pH 7 and above. The imidazolidinone ring was opened to form an intermediate (RP 35606) which formed an isomer of iprodione (RP 30228), which is further degraded slowly.

Iprodione did not show light absorption above 290 nm, so under aqueous photolysis conditions no significant influence of light was determined. A water/sediment study gave whole system $DegT_{50}$ values of 7.3 and 6.3 days for iprodione and 87.7 and 45.0 days for RP 30228 from river and pond systems respectively.

Several hydrolysis studies were performed with iprodione and its metabolites RP 35606 and RP 30228 to understand the pH dependency of the equilibrium.

An aerobic mineralisation in surface water study, which is a new data requirement under 1107/2009, was also conducted. Natural pond water was utilised to investigate the biodegradability of iprodione. Degradation occurred as expected, therefore a kinetic evaluation of the active could be performed from be performed.

In order to support the analytical method a storage stability study for RP 30228 was performed.

(Not yet peer-reviewed study)

Report:	CA 7.2/1 Schulz H.,Meyer M., 2007a Determination of the storage stability of RP 30228 in surface water 2007/1039574
Guidelines:	Chemikaliengesetz of 25th July 1994, OECD-DOC ENV/MC/CHEM(98)17 Paris 1998, EEC 7032/VI/95 rev. 5
GLP:	Yes (certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Executive Summary

The storage stability of RP 30228 in surface water at the storage temperature of ≤ -18°C was determined by means of the recovery rates in residue analyses. The storage stability was checked after storage periods of up to six months.

Untreated specimen material was fortified with RP 30228 at a fortification level of 5 µg/kg to obtain the storage stability specimens. On each date of analysis, one control specimen, two storage stability specimens and one freshly prepared fortified specimen were analysed.

The recovery values in % of RP 30228 obtained from the storage stability specimens at the seven analysis dates (i.e. over the whole storage time period) ranged from 85% to 104%.

The recovery values were not corrected by the mean recovery values of the concurrent analysed freshly prepared fortified specimens.

The correctness of the analytical method was demonstrated by simultaneous analysis of a freshly prepared fortified specimen on each date of analysis. The fortification level was the same as for the storage stability specimens. The mean value of these recovery rates was 98.3 % and the coefficient of variations (CV) was 7.4 %. The limit of quantification of the analytical method was 0.05 µg/kg.

The storage stability of RP 30228 in surface water could be confirmed over a period of 6 months.

I. MATERIAL AND METHODS

A. MATERIALS

Test materials: RP 30228
Purity: 99.8 % w/w

B. STUDY DESIGN

4. Experimental Conditions

The surface water was obtained from a stream in the municipal of Dietzhölztal-Ewersbach in a drinking water reserve without farming. The concentration of RP 30228 in this surface water was < 0.05 µg/kg.

The storage stability specimens were fortified at approximately 5 µg/kg of RP 30228 (100 x LOQ). About 30 g of the control specimens were weighed into a 40 ml plastic bottle. A calculated volume of RP 30228 standard solution was added in small quantities to the control specimens. The plastic bottles were closed and stored deep-frozen at ≤ -18°C. Time zero specimens were extracted immediately after preparation without prior deep-freezing.

The storage stability tests were performed in 7 analytical assays, in intervals of 0 days, 7 days, 14 days, 1 month, 2 months, 4 months and 6 months after the preparation of the specimens. For each analysis date one control specimen (untreated), two storage stability specimens and one freshly prepared fortified specimen were used.

The water specimens to be used for the preparation of the freshly prepared fortified specimens were stored deep-frozen without treatment.

5. Description of analytical procedures

The analytical method used in this study was developed and validated at the testing facility SGS Institute Fresenius GmbH, Taunusstein – Germany.

The analyte was extracted from water using dichloromethane. After concentration to dryness and reconstitution, the final determination was performed by HPLC-MS/MS using the mass transitions m/z 330.0 → 100.8 and m/z 332.0 → 100.8. The limit of quantification of the method was 0.05 µg/kg.

II. RESULTS AND DISCUSSION

The correctness of the analytical methods was proven by simultaneous analysis of a freshly prepared fortified specimen on each date of analysis. Seven surface water specimens were fortified at the same fortification level used for the storage stability specimens (~5 µg/kg). The mean value of these recovery rates was 98.3 % with a coefficient of variation (CV) of 7.5 %.

The storage stability of RP 30228 in surface water at the storage temperature of < -18°C was determined by means of the recovery rates in residue analyses. The recovery values in percentage of RP 30228 at the seven analysis dates (i.e. over the whole storage time period) are summarised in Table 7.2-1.

Table 7.2-1: Results of the storage stability of RP 30228

Storage period [days / months]	Residues [µg/kg]		Recovery [%]
	Nominal	Actual	
0 days	5.1233	4.6071	90
0 days	5.1165	5.1318	100
7 days	5.1199	4.8711	95
7 days	5.1199	5.2538	103
14 days	5.1216	4.8243	94
14 days	5.1199	4.9177	96
1 month	5.1216	4.4389	87
1 month	5.1199	4.3739	85
2 months	5.1199	5.0750	99
2 months	5.1199	5.1498	101
4 months	5.1182	4.9247	96
4 months	5.1199	5.0483	99
6 months	5.1114	5.1601	101
6 months	5.1165	5.3307	104

III. CONCLUSION

The storage stability of RP 30228 in surface water was confirmed over a period of 6 months.

CA 7.2.1 Route and rate of degradation in aquatic systems (chemical and photochemical degradation)

CA 7.2.1.1 Hydrolytic degradation

Studies presented in the original Annex II Dossier (1998) and summarized in this supplementary dossier to provide a complete view on the fate of iprodione in the environment:

Report: Das, Y.T. (1990): Hydrolysis of [phenyl-(U)-^{14}C]-iprodione in aqueous solutions buffered at pH 5, 7 and 9.
Report ISSI no 89100 of December 31, 1990.
R014577
GLP: Yes

A study was carried out in the USA to comply with the EPA requirement and in compliance with Good Laboratory Practice Standards. Sterile deionised water buffered at pH 5, pH 7 or pH 9 was dosed with the test compound at a concentration of 11.0 ppm (pH 5), 12.3 ppm (pH 7), 12.0 ppm (pH 9; replication 1) or 12.3 ppm (pH 9; replication 2). The test solutions were contained in individual test vessels and were incubated in the dark for up to 30 days at 25 ± 1°C.

Test vessels were sampled at different times and analysed by HPLC for the parent and hydrolytic products. The identity of the parent and its degradates was confirmed by LC-MS. Two test vessels were sampled at various times after the treatment:

Results: The test solutions were found to maintain their buffering capacity and sterility for the study period. Material balance was 99.0 ± 1.6% for pH 5, 101.1 ± 0.6% for pH 7 and 100.5 ± 1.2% for pH9.

Iprodione was found to be relatively stable in buffered aqueous solutions at pH 5 maintained under dark conditions at 25 ± 1°C. Its concentration dropped from an initial value of 96.1% to a final value of 81.6% by 30 days. The only major degradate, RP 35606, increased from 0.4% to 11.4%.

At pH 7 iprodione decreased from an initial value of 92.2% to a final value of 41.0% by 125 h. RP 35606 reached a maximum concentration of 10.1% by 40 min. In addition to RP 35606, a second major degradate, RP 30228, was identified. This degradate reached a maximum concentration of 45.6% by 125 h. One unidentified compound reached a maximum of 8.2% after 17 hours and declined to 4.2% after 125 hours. As RP 36221 and RP 32490 were also tested as reference compounds, these metabolites could be excluded.

At pH 9 iprodione decreased rapidly to 4.5% by 114 min. RP 30228, the only major degradate, reached a maximum concentration of 92% by 114 min. RP 35606 reached a maximum concentration of 1.7% at 107 min. The data are summarised in tables shown below.

The calculated half-lives of iprodione at 25°C were 130.7 days at pH 5, 6.4 days at pH 7 and 27.2 min at pH 9.

Table 7.2.1.1-1: Iprodione and its degradates in pH 5 test solutions [mean % TAR]

Time [days]	Iprodione	RP 35606	Others*
0	96.1	0.4	3.5
1	95.4	0.4	4.1
3	94.7	1.2	4.1
7	89.9	3.2	7.0
14	88.2	6.0	5.8
21	86.0	9.1	4.9
30	81.6	11.4	7.1

* sum of several insignificant radioactive areas

Table 7.2.1.1-2: Iprodione and its degradates in pH 7 test solutions [mean % TAR]

Time [hours]	Iprodione	RP 35606	RP 30228	Others*
0	92.2	0.2	2.2	5.3
5.3	87.2	0.4	4.5	7.8
17.0	70.9	2.7	13.0	13.5
40.4	56.7	10.1	23.2	10.0
76.0	54.3	5.7	28.3	11.6
124.7	41.0	15.0	45.6	8.2

* sum of three unknowns and several insignificant radioactive areas

Table 7.2.1.1-3: Iprodione and its degradates in pH 9 test solutions [mean % TAR]

Time [min.]	Iprodione	RP 35606	RP 30228	Others*
0	87.6	<0.1	9.7	2.8
14/15	53.9	0.1	42.6	3.6
29	35.5	0.1	60.6	3.9
45/50	22.2	0.4	74.8	2.7
60/65	14.1	0.6	83.2	2.2
107/121	4.5	1.6	92.0	2.1

* sum of several insignificant radioactive areas

A second hydrolysis study with iprodione was performed with an extended range of reference compounds for structure identification by Völkel [*Völkel (1997) – R003478*]. In addition a more detailed investigation into the equilibrium between iprodione, RP 35606 and RP 30228 was done by two studies of Mamouni [*Mamouni (1999a) – C022878; Mamouni (1999b) – C022850*], where the metabolites were used as starting material. These studies were discussed in detail by Leake & Buntain [*Leake & Buntain (2000) – C022972*].

Report: Völkel, W. (1997): Hydrolysis of ^{14}C-iprodione at different pH values. Report RCC no 667620 of December 30, 1997. R003478
GLP: Yes

Hydrolysis was investigated with [^{14}C]-phenyl ring labelled iprodione in aqueous solutions of pH 5, 6, 7 and 8 at 25 ± 1°C over a 32 day period.
The experiment was set up first by incubating individual samples in duplicate for each time point. Each sample consisted of 10 mL sterile buffer solution containing the test substance in tightly closed vessels placed in a shaking water bath. During the incubation (32 days), 9 samples for pH 5 and 6 and 12 samples for pH 7 and 8 were taken and analysed by HPLC. The temperature was constant throughout the incubation period (25.6 ± 0.1°C). No significant variation of the pH values was observed in the buffered solutions.

Results: Recoveries of total radioactivity were in the range of 93.5% to 102.7% of initial radioactivity at each sampling interval.
The amount of iprodione decreased from the initially applied 98.7%, 98.2%, 97.4% and 96.7% of the radioactivity on day 0, to 84.7%, 40.1%, 2.6% and 0.9% on day 32, in the buffer solutions of pH 5, 6, 7 and 8, respectively.

The main hydrolysis product after 32 days of incubation was RP 30228 which amounted to 13.9%, 51.9%, 94.3% and 97.8%, respectively for buffer solutions of pH 5, 6, 7 and 8.
An intermediate reaction product was detected, i.e. RP 35606. In the solution at pH 5 its quantity amounted to 1% of the radioactivity applied. However, in the solution at pH 6 it amounted to a maximum of 6.9% after 4 days of incubation. In the solution at pH 7 it reached a maximum of 35.0% on day 2 and in solution at pH 8, it amounted to 67.3% on day 1.
Metabolite RP 32596 (i.e. 3.5-dichloroaniline was detected only in the buffer solution pH 8 in small amounts not exceeding 0.3% of the radioactivity applied.

Conclusion: The hydrolysis of iprodione consisted in a rearrangement, with ring-opening of the parent molecule, formation of the intermediate substance RP 35606 and ring closure to form RP 30228. In addition, RP 32596 *i.e.* 3,5-dichloroaniline was detected in small amounts not exceeding 0.3% of the applied radioactivity at pH 8.

The rate of degradation of iprodione was highly pH dependent with half-life values ranging from 0.2 to 146 days (see Table 7.2.1.1-4).

Table 7.2.1.1-4: Rate of degradation of iprodione at different pH values (25°C)

	pH 5	pH 6	pH 7	pH 8
DT_{50} [days]	146	25	3	0.2
DT_{90} [days]	487	83	10	0.8

Report: Mamouni, A. (1999a): [^{14}C]-RP 35606 (intermediate hydrolysis product of iprodione). Hydrolysis at four different pH values.
Report RCC no 732826 of December 20, 1999.
C022878
GLP: Yes

The hydrolysis of [^{14}C]-phenyl ring labelled RP 35606 has been investigated in aqueous solutions at pH 4, 7, 8 and 9. A range of temperatures was investigated including the standard 50°C and 25°C as given in the "Modified OECD Guideline for Testing Chemicals 111 (Hydrolysis as function of pH) adopted May 12, 1981" and, in addition, 13.5°C and 40°C. The rate of hydrolysis of RP 35606 as well as the formation of hydrolysis products were investigated for up to 50 days.

Due to the instability of RP 35606, it was prepared by hydrolysis of the parent compound iprodione in a special buffer solution to ensure > 95% RP 35606 at commencement of the experiment.

The experiment was set up by incubating individual samples for each time point. Each sample consisted of 10 mL of sterile buffer solution containing the test compound in tightly closed vessels placed in a shaking water bath or in an incubator using a magnetic stirrer for mixing.

During the incubation and for each pH, 8 samples for 13.5°C, 9 samples (duplicate) for 25°C, 6 samples for 40°C and 3 samples (duplicate) for 50°C were taken and analysed by HPLC. Selected samples were also analysed by TLC. The temperature was constant throughout the incubation period (13.5 ± 0.4°C, 25.1 ± 0.3°C, 40 ± 0.5°C and 49.7 ± 0.3°C).

Results: No significant variation of the pH values was observed in the buffered solutions during the incubation. The mean recoveries of total radioactivity were in the range of 95.1 % to 103.7 % of initial radioactivity.

[^{14}C]-RP 35606 and selected radioactive fractions were characterised by co-elution with certified reference substances by HPLC-analysis and by co-chromatography using TLC. The quantitative determination was carried out based on the results of the HPLC-analysis.

The rate of degradation of RP 35606 was highly pH dependent with half-life values ranging from 0.5 hours to greater than 50 days (see Table 7.2.1.1-5).

Table 7.2.1.1-5: DT_{50} values of RP 35606

	DT_{50} [days]											
	13.5°C				**25°C**				**40°C**			
	pH 4	**pH 7**	**pH 8**	**pH 9**	**pH 4**	**pH 7**	**pH 8**	**pH 9**	**pH 4**	**pH 7**	**pH 7**	**pH 9**
Incubation [days]	**50**				**50**				**29**			
RP 35606	0.02*	3.0	8.0	>50	0.02*	1.1	2.1	27.9	0.02*	0.7**	0.6	5.8

* determined experimentally to be approximately 0.5 hours at room temperature
** extrapolated

At low pH (pH 4), "hydrolysis" of RP 35606 proceeded rapidly at all temperatures with the reformation of iprodione which is actually a loss of H_2O.
At temperatures of 13.5°C and 25°C the iprodione formed was stable, with virtually complete conversion. After 50 days there was limited evidence for the presence of RP 30228 with 3.7% (13.5°C), 4.9% (25°C) and 14.2% (29 days at 40°C). Although it appeared that RP 30228 is being formed directly from iprodione at pH 4 at 40°C it is possible that the rate of formation of RP 30228 from RP 35606 was so much more rapid than the rate of RP 35606 that no RP 35606 is observed. There is no clear explanation as to why this occurs without passing through the intermediate compound RP 35606.

At pH 7 "hydrolysis" of RP 35606 proceeded more steadily than at pH 4 with the formation of both iprodione and RP 30228. At this pH any iprodione formed was unstable, such that all temperatures the terminal product formed is RP 30228, which is stable at pH 7. The quantity of iprodione formed was temperature dependent with up to 34% at 13.5°C, 30% at 25°C and 28% at 40°C. The rate of transformation of iprodione was also temperature dependent being more rapid at higher temperature.

At pH 8 "hydrolysis" of RP 35606 proceeded more slowly than at pH 4 or 7, with the formation of both iprodione and RP 30228. In addition three other compounds were observed, RP 36233 and RP 37176 together with 3,5-dichloroaniline. The maximum quantity of iprodione detected was lower than at pH 7 and did not exceed 10%. This was consistent with the more rapid transformation of iprodione under alkaline conditions, resulting in the formation of RP 30228. However, at this pH RP 30228 further degraded with the formation of RP 37176 (up to 20% at 40°C), RP 36233 (up to 7.0% at 40°C) and 3, 5-dichloroaniline (16.5% at 40°C).

At pH 9 "hydrolysis" of RP 35606 proceeded the slowest of all the pH values. At this pH iprodione was not detected although its role as a transitory intermediate was evident by the appearance of the hydrolysis product RP 37176.
The transformation product RP 30228 was rapidly formed, the rate of formation being influenced by temperature. However, RP 30228 was also rapidly degraded at this pH, particularly at the higher temperatures. The main degradation product formed was RP 36233, which reached a maximum of 52% at 40°C. Also formed was RP 37176, which reached a maximum of ca. 13% at 40°C together with 3,5-dichloroaniline, which can be formed from both RP 36233 and RP 37176 and reached a maximum of ca. 22%.

Conclusion: The pH of the test system had two separate effects upon the conversion of RP 35606. Firstly a pH dependent equilibrium exists between iprodione and RP 30228 via the intermediate RP 35606. At pH 4 the equilibrium lies towards iprodione, whereas at pH 7, 8 and 9 it lies towards RP 30228. The rate of conversion from RP 35606 is fastest at pH 4 and slows with increasing pH from pH 7 to 9. The DT_{50} (1st order) values at 25°C for RP 35606 range from ca. 0.5 hours (0.02 days) at pH 4 to 1.1. days at pH 7 and 2.1 days at pH 8, rising to 27.9 days at pH 9.

The second effect of pH was the rate at which other hydrolytic products were formed from this equilibrium mixture of iprodione, RP 35606 and RP 30228.
At pH 4 and 7 there was no further hydrolysis since iprodione is stable at pH 4 and RP 30228 is stable at pH 7.
At pH 8 and 9 there was further hydrolysis to RP 37176 and RP 36233 and ultimately to 3,5-dichloroaniline. The rate of this hydrolysis was apparently faster at the higher pH values.

The proposed hydrolysis pathway is presented in Figure 7.2.1.1-1:

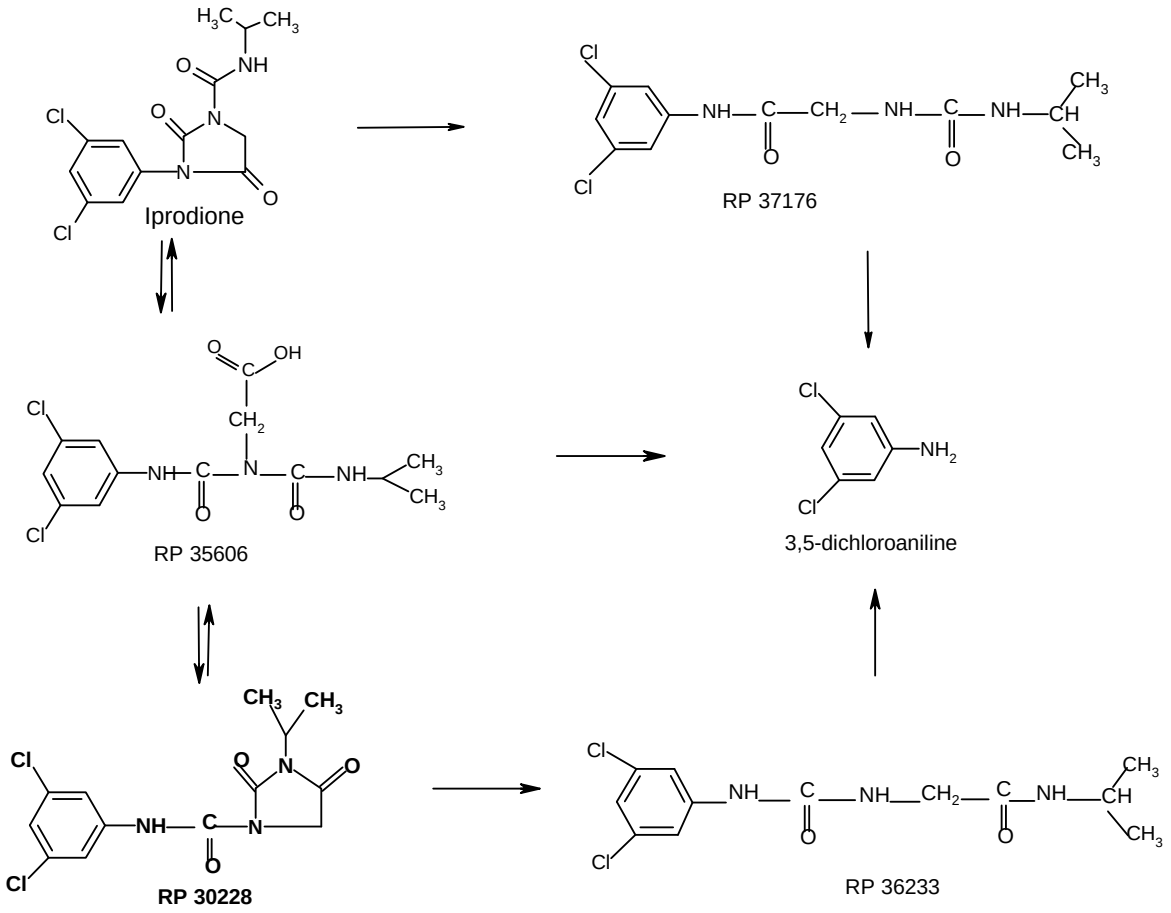


Figure 7.2.1.1-1: Proposed hydrolysis pathway of iprodione in water/sediment systems

While the degradation to dichloroaniline proceeds via ring open intermediates, it is not very likely that significant amounts of the hydantoine RP 30181 will be formed. The stepwise degradation of the side chain is the most likely pathway.

Report: Mamouni, A. (1999b): [^{14}C]-RP 30228 (metabolite of iprodione). Hydrolysis at four different pH values and investigation on possible reformation of iprodione.
Report RCC no 732824 of December 3, 1999.
C022850
GLP: Yes

A study has been conducted to investigate the hydrolysis of RP 30228, metabolite of iprodione, at 4 pH values and to look into the possibility of reformation of iprodione.
The hydrolysis of [^{14}C]-phenyl ring labelled RP 30228 was carried at pH 4, 7, 8 and 9 at 50 ± 0.3°C, over a 5 day period.

The experiment was conducted by incubating individual samples in duplicate for each time point. Each sample consisted of 10 mL sterile buffer solution containing the test compound in tightly closed vessels placed in an incubator under stirring. During the incubation (5 days), 5 samples for pH 4, 7, 8 and 9 were taken and analysed by HPLC.

Results & conclusion: The temperature remained constant throughout the incubation period (50 ± 0.3°C) and no significant variation of the pH values was observed in the buffered solutions.
Mean recoveries of total radioactivity were in the range of 90.2 % to 99.3 % of initial radioactivity at each sampling interval.

At pH 4 and pH 7 RP 30228 was not hydrolysed at 50°C within the incubation time of 5 days.

At pH 8 and pH 9, RP 30228 was rapidly hydrolysed, hydrolysis being faster in the pH 9 buffer solution compared to pH 8. RP 30228 decreased from 100.0 % of the applied radioactivity on day 0 to 57.9 % after 1 day of incubation. After 5 days on incubation, RP 30228 was only detected at pH 8 amounting to 31.6 %.
It should be stated that the sample at pH 9 was analysed (74.7 % applied radioactivity) about 2 hours after application of the test compound to the buffer solution. After 1 day, the quantity of RP 30228 had decreased to 10.7 % at pH 9. Thus, it can be seen that the hydrolysis at pH 9 is more rapid than at pH 8.

DT_{50} value of hydrolysis of RP 30228 at pH 8 was calculated to be 1.8 days. At pH 9, the corresponding value was 0.3 days by considering the amount of RP 30228 at time 0 as 100 %.
[^{14}C]-RP 30228 was hydrolysed to RP 35606 which was then further degraded. Also formed were the two degradation products RP 36233 and RP 37176, followed by further degradation to 3,5-dichloroaniline.
RP 35606 was detected as an intermediate reaction product. In the solution at pH 8, its quantity amounted to 26.0 % of the radioactivity applied after 1 day and then declined to 12.7 % after 5 days of incubation.
Besides RP 35606, a small amount of iprodione (2.5 %) was detected after 1 day of incubation only and only at pH 8. After this time, the amount of iprodione rapidly dispersed. The quantity of the other radioactive components increased continuously with RP 36233, RP 37176 and 3,5-dichloroaniline amounting to 7.7 %, 23.1 % and 24.1 % after 5 days of incubation, respectively.

At pH 9, RP 35606 reached a maximum of 47.5 % of the radioactivity applied after 1 day and was no longer detected at day 5. Iprodione was not detected in any of the analysed samples.
The quantity of the other radioactive components RP 36233, RP 37176 and 3,5-dichloroaniline increased continuously amounting to 47.1 %, 13.6 % and 38.3 % after 5 days of incubation, respectively.

Table 7.2.1.1-6: Residues of iprodione and its metabolites at different pH values

50°C	Values in % of applied radioactivity											
Balance of applied radio-activity	Sampling intervals [days]											
	pH 4			pH 7			pH 8			pH 9		
	0	1	5	0	1	5	0	1	5	0*	1	5
RP 30228	101.6 98.4	91.0 90.3	99.5 98.3	100.8 99.2	90.9 89.5	98.1 95.5	99.2 100.8	55.1 60.8	32.2 31.1	74.7 74.7	10.2 11.2	n.d. n.d.
Mean	100.0	90.6	98.9	100.0	90.2	96.8	100.0	57.9	31.6	74.7	10.7	n.d.
Iprodione	n.d. n.p.	n.d. n.d.	n.d. n.d.	n.d. n.d.	n.d. n.d.	n.d. n.d.	n.d. n.p.	2.2 2.8	n.d. n.d.	n.d. n.d.	n.d. n.d.	n.d. n.d.
Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.5	n.d.	n.d.	n.d.	n.d.
RP 35606	n.d. n.p.	n.d. n.d.	n.d. n.d.	n.d. n.p.	n.d. n.d.	n.d. n.d.	n.d. n.p.	24.8 27.2	12.8 12.6	26.1 n.p.	49.7 45.2	n.d. n.d.
Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	26.0	12.7	26.1	47.5	n.d.
RP 36233	n.d. n.p.	n.d. n.d.	n.d. n.d.	n.d. n.p.	n.d. n.d.	n.d. n.d.	n.d. n.p.	n.d. n.d.	7.8 7.6	n.d. n.p.	18.1 21.5	46.8 47.4
Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.7	n.d.	19.8	47.1
RP 37176	n.d. n.p.	n.d. n.d.	n.d. n.d.	n.d. n.p.	n.d. n.d.	n.d. n.d.	n.d. n.p.	3.5 4.2	23.3 22.9	n.d. n.p.	5.2 6.2	13.5 13.7
Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.8	23.1	n.d.	5.7	13.6
3,5-dichlor-aniline	n.d. n.p.	n.d. n.d.	n.d. n.d.	n.d. n.p.	n.d. n.d	n.d. n.d.	n.d. n.p.	7.0 6.3	22.5 25.7	n.d. n.p.	14.1 12.3	38.8 37.9
Mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.7	24.1	n.d.	13.2	38.3

n.p. = not performed
n.d. = not detected
This sample was analysed about 2 hours before addition of the test compound to the buffer solution.

Conclusion: The hydrolysis of RP 30228 was highly influenced by the pH, with the rate of hydrolysis increasing with increasing pH. RP 30228 was stable at pH 4 and 7 even at 50°C and no hydrolysis or degradation took place over the incubation period of 5 days.
However, under alkaline conditions at pH's 8 and 9, RP 30228 was rapidly hydrolysed with DT_{50} values of 1.8 and 0.3 days, respectively.
The pathway followed two directions, the direct degradation to RP 36233 and the degradation to RP 37176 via the hydrolysis product RP 35606 and iprodione but with the rate of iprodione degradation so rapid that it was not observed.
The degradation products RP 36233 and RP 37176 are then degraded to 3,5-dichloroaniline.

Virtually no iprodione (< 3%) was detected indicating that hydrolysis is not a major contributor to the re-formation of iprodione or that any iprodione formed is so rapidly further hydrolysed under alkaline conditions that it is not observed.

The proposed hydrolysis pathway is presented in Figure 7.2.1.1-1.

The effect of pH is therefore of vital importance to transformations taking place between the three compounds iprodione, RP 35606 and RP 30228. In addition, the pH determines which route the degradation to RP 37176 and RP 36233 will preferentially follow.

The influence of temperature at each pH value in general has a significant effect on the rate of reaction with, as expected, an increased rate with increased temperature. This can result in the rapid degradation of intermediate products such that they are not actually observed. Thus, it is possible that at 25°C at pH 8 and pH 9 hydrolysis of RP 30228 might result in the re-formation of iprodione. However, the evidence from the iprodione study at 25°C and pH 8 suggests that the equilibrium lies in the direction of RP 30228 with no iprodione present**.** Additionally, at both pH 8 and pH 9, the RP 35606 study shows that the equilibrium lies in the direction of RP 30228. Thus, it is considered that the study conducted at 50°C with RP 30228 is sufficient to address this issue when taken in context of the studies with iprodione and RP 35606.

The results from the three studies listed above [*Völkel (1997) – R003478; Mamouni (1999a) – C022878; Mamouni (1999b) – C022850]* are summarised in Leake & Buntain [*Leake & Buntain (2000) – C022972*] as given below.

Report: CA 7.2.1.1/1
Leake C.R.,Buntain I.G., 2000b
The hydrolysis of Iprodione, RP 35606 and RP 30228 at a range of pH values and temperatures
C022972
Guidelines: none
GLP: no

Executive Summary

The hydrolysis of iprodione, RP 35606 and RP 30228 was separately investigated at a range of different pH values and temperatures.

The results showed consistent pH dependence for the inter-conversion between iprodione, RP 35606 and RP 30228. Iprodione hydrolysed more rapidly under alkaline conditions compared to acidic conditions. The rate of degradation of iprodione was highly pH dependent with half-life values ranging from 0.2 to 146 days.

Commencing with the ring opened hydrolysis product RP 35606 the equilibrium at pH 4 lies towards iprodione whereas at pH 7, 8 and 9 it lies towards RP 30228. At pH 4 iprodione was stable so no further degradation takes place and at pH 7 RP 30228 was stable. Thus, only at pH 8 and 9 did further degradation at both ends of the equilibrium take place.

RP 30228 was stable at pH 4 and 7 and no hydrolysis or degradation took place.

At alkaline pH levels RP 30228 degraded either directly to RP 36233 or to RP 37176 via RP 35606 and iprodione. The degradation products RP 36233 and RP 37176 were then degraded to 3,5-dichloroaniline.

The influence of temperature at each pH value in general had a significant effect on the rate of reaction. As expected, an increased rate with increased temperature. This can result in the rapid degradation of intermediate products such that they are not actually observed.

I. MATERIAL AND METHODS

1. Experimental conditions

The hydrolysis of iprodione, RP 35606 and RP 30228 was separately investigated at a range of different pH levels and temperatures.

The hydrolysis of [^{14}C]-phenyl ring labelled iprodione was investigated in aqueous solutions of pH 5, 6, 7 and 8 at 25°C ± 1°C over a 32 day period [*Völkel (1997) – R003478*].

The hydrolysis of [^{14}C)-phenyl ring labelled RP 35606 was investigated in aqueous solutions at pH 4, 7, 8 and 9 [*Mamouni (1999a) – C022878*]. A range of temperatures was investigated, the standard 50°C and 25°C and, in addition, 13.5°C and 40°C. Due to the instability of RP 35606 it was prepared by hydrolysis of iprodione in a special buffer solution to ensure >95% RP 35606 at commencement of the experiment.

The hydrolysis of [^{14}C]-phenyl ring labelled RP 30228 was investigated in aqueous solutions at pH 4, 7, 8 and 9 [*Mamouni (1999b) – C022850*]. The study was conducted at 50 ± 0.3°C.

2. Sampling

For iprodione hydrolysis experiments, samples were incubated for 0, 1, 4, 7, 11, 15, 20, 26 and 32 days at pH 5 and 6. At pH 7 and 8 the incubation intervals were 0, 0.17, 0.25, 1, 2, 4, 7, 11, 15, 20, 26 and 32 days.

For RP 35606 hydrolysis experiments, samples were incubated at 13.5 and 25°C for 0, 1, 2, 5, 9, 13, 33, and 50 days at all pH levels. At 25°C, an additional sample was taken after 23 days. At temperature 40°C, samples were incubated for 0, 1, 5, 12, 22 and 29 days for all pH levels.

For the hydrolysis of RP 30228, samples were incubated for 0, 1, and 5 days.

II. RESULTS AND DISCUSSION

Iprodione

Iprodione was hydrolysed at pH 5, 6, 7 and 8. The rate of hydrolysis was pH dependent being much more rapid under alkaline conditions. Iprodione hydrolysed by ring opening and the addition of H_2O to form RP 35606 followed by dehydration to form the transposition product RP 30228. The distribution of radioactivity during hydrolysis of iprodione at various pH values is shown in Table 7.2.1.1-7 to Table 7.2.1.1-9.

A good total recovery of radioactivity was obtained for the hydrolysis of [^{14}C]-phenyl ring labelled iprodione, ranging from 93.5% to 102.7%.

At pH 5 the hydrolysis of iprodione proceeded steadily. The metabolite RP 30228 was the only major product being formed while a very small amount of the metabolite RP 35606 was present throughout (≤ 1%).

At pH 6 iprodione was hydrolysed slightly more rapidly than at pH 5. The quantity of RP 35606 increased compared with pH 5 but still remained below 10 %. There was clearer evidence for formation and decline of RP 35606. The quantity of the transposition product RP 30228 was increased when compared with pH 5 resulting in the formation of up to 52 % at day 32. There was no evidence of any decline of RP 30228. Three other minor products were also observed, however, none of them exceeded 1%.

Table 7.2.1.1-7: Distribution of ^{14}C-iprodione and formation of hydrolysis products during incubation in buffer solution at pH 5 and 6. Results are mean of duplicate samples in % TAR

DAT	Iprodione	RP 35606	RP 36233 / RP 37176	RP 30228	M7 (unknown)	M8 (unknown)
pH 5						
0	98.7	0.1	0.3	0.0	0.6	0.5
1	91.4	0.8	0.3	0.1	0.3	0.5
4	92.8	1.0	0.3	1.5	0.7	0.6
7	94.6	0.4	n.d.	3.4	0.5	0.4
11	90.9	0.4	n.d.	6.4	0.3	0.7
15	88.1	0.6	n.d.	7.6	0.7	0.3
20	86.3	0.6	0.4	7.7	0.6	0.5
26	81.5	0.7	0.3	11.2	n.d.	n.d.
32	84.7	0.4	0.2	13.9	0.4	0.2
pH 6						
0	98.2	0.4	0.4	0.0	0.6	0.6
1	87.6	4.7	0.3	0.9	0.5	0.6
4	84.1	6.9	0.5	6.5	0.5	0.6
7	75.0	5.2	0.2	15.0	0.6	0.2
11	66.7	4.7	0.3	23.2	n.d.	0.2
15	61.1	3.9	0.7	28.6	0.6	0.2
20	53.4	3.2	0.6	38.0	0.4	n.d.
26	45.8	3.1	1.0	48.2	0.2	0.2
32	40.1	2.7	0.8	51.9	0.1	n.d.

At pH 7 the hydrolysis of iprodione proceeded more rapidly than at pH 5 or 6. The quantity of RP 35606 increased compared to that present at pH 5 and 6 reaching a maximum of about 35% and then readily declining.

There was a steady increase in the formation of the transposition product RP 30228 with an indication that the material formed RP 35606 first, followed by ring closure to form RP 30228. The quantity of RP 30228 reached a maximum of about 95% showing almost total conversion of iprodione at this pH level.

Table 7.2.1.1-8: Distribution of ^{14}C-iprodione and formation of hydrolysis products during incubation in buffer solution at pH 7. Results are mean of duplicate samples in % TAR

DAT	Iprodione	RP 35606	RP 36233 / RP 37176	RP 30228	M7 (unknown)	M8 (unknown)
0	97.4	0.6	0.5	0.0	1.0	0.6
0.17	90.3	4.7	0.2	0.5	0.5	0.2
0.25	81.5	13.4	0.2	0.5	0.7	0.6
1	62.1	29.3	0.5	4.6	0.3	0.6
2	49.6	35.0	0.5	13.1	0.5	n.d.
4	38.4	25.9	0.8	37.2	0.3	n.d.
7	24.2	16.5	0.6	55.9	n.d.	n.d.
11	10.6	8.9	1.0	76.6	n.d.	n.d.
15	8.6	5.3	1.8	81.7	n.d.	n.d.
20	4.1	1.7	1.3	87.5	n.d.	n.d.
26	2.6	0.9	1.4	91.6	n.d.	n.d.
32	2.6	0.5	1.5	94.3	n.d.	n.d.

At pH 8 the hydrolysis of iprodione proceeded even more rapidly than at pH 5, 6 or 7. The quantity of RP 35606 increased compared to that present at pH 5, 6, and 7, reaching a maximum of about 60% before readily declining.

Table 7.2.1.1-9: Distribution of ^{14}C-iprodione and formation of hydrolysis products during incubation in buffer solution at pH 8. Results are mean of duplicate samples in % TAR

DAT	Iprodione	RP 35606	RP 32247	RP 36233 / RP 37176	RP 32596	RP 30228	M7 (unknown)	M8 (unknown)
0	96.7	2.0	n.d.	0.3	n.d.	n.d.	0.7	0.4
0.17	72.1	18.3	n.d.	0.7	n.d.	1.5	0.6	0.4
0.25	35.9	54.0	0.2	0.4	n.d.	2.9	0.4	0.3
1	11.7	67.3	0.4	0.7	n.d.	13.6	n.d.	0.1
2	5.0	60.8	n.d.	1.1	n.d.	35.2	n.d.	n.d.
4	2.6	33.4	n.d.	1.6	0.2	69.8	n.d.	n.d.
7	2.4	11.7	n.d.	1.2	n.d.	80.1	n.d.	n.d.
11	0.7	4.9	n.d.	1.6	0.2	89.9	n.d.	n.d.
15	1.4	1.8	n.d.	1.2	n.d.	94.5	n.d.	n.d.
20	0.9	0.8	n.d.	1.7	0.2	90.8	n.d.	n.d.
26	1.1	0.7	n.d.	1.6	0.3	91.9	n.d.	n.d.
32	0.9	0.5	n.d.	1.4	n.d.	97.8	n.d.	n.d.

As at pH 7, there was a steady increase in the formation of the transposition product RP 30228 with an indication that the material formed RP 35606 first, followed by ring closure to form RP 30228. The quantity of RP 30228 reached a maximum of about 98% showing almost total conversion of iprodione at this pH value.

The rate of degradation of iprodione in buffer solution was highly pH dependent with half-life values ranging from 0.2 to 146 days. A summary of iprodione degradation rates at various pH levels is given in Table 7.2.1.1-10.

Table 7.2.1.1-10: Iprodione degradation rate in buffer solution at various pH range

pH	DT_{50} [days]	DT_{90} [days]
5	146	487
6	25	83
7	3	10
8	0.2	0.8

RP 35606

The hydrolysis of RP 35606 in aqueous solutions was investigated at pH 4, 7, 8, and 9. A range of temperatures was used; the standard 50°C and 25°C and in addition, 13.5°C and 40°C. The distribution of radioactivity during hydrolysis of RP 35606 at various pH values and various temperatures is given in Table 7.2.1.1-11 to Table 7.2.1.1-14.

The total recovery values for the hydrolysis of [^{14}C]-phenyl ring labelled RP 35606 ranged from 95.1 to 103.7%.

The hydrolysis of RP 35606 at pH 4 proceeded rapidly at all temperatures with the reformation of iprodione by a loss of H_2O. At temperatures of 13.5°C and 25°C the iprodione formed was stable, with virtually complete conversion. There was limited evidence for the presence of RP 30228 after 50 days with 3.7% at 13.5°C, 2.5% at 25°C and 14.2% after 29 days at 40°C.

Although it appears that RP 30228 is being formed directly from iprodione at pH 4 at 40°C it is possible that the rate of formation of RP 30228 from RP 35606 is so much more rapid than the rate of RP 35606 that no RP 35606 is observed. There is no clear explanation as to why this occurs without passing through the intermediate compound RP 35606.

Table 7.2.1.1-11: Distribution of ^{14}C-RP 35606 and formation of hydrolysis products during incubation in buffer solution at pH 4 and various temperatures. Results are in % TAR

DAT	RP 35606	Iprodione	RP 30228
pH 4 / 13.5°C			
0	73.0	27.0	n.d.
1	n.d.	98.3	n.d.
2	n.d.	98.8	n.d.
5	n.d.	89.4	6.1
9	n.d.	99.9	n.d.
13	n.d.	99.9	n.d.
33	n.d.	101.9	n.d.
50	n.d.	91.8	3.7
pH 4 / 25°C*			
0	73.0	27.0	n.d.
1	n.d.	97.9	n.d.
2	n.d.	95.6	3.3
5	n.d.	97.4	1.5
9	n.d.	99.0	1.0
13	n.d.	98.5	n.d.
23	n.d.	99.4	n.d.
33	n.d.	99.1	n.d.
50	n.d.	92.3	2.5
pH 4 / 40°C			
0	73.0	27.0	n.d.
1	n.d.	99.5	n.d.
5	n.d.	98.2	n.d.
12	n.d.	99.9	n.d.
22	n.d.	92.7	7.1
29	n.d.	84.6	14.2

* values are average of duplicate sampling
n.d. = not detected

At pH 7 the hydrolysis of RP 35606 proceeded more steadily than at pH 4 with the formation of both iprodione and RP 30228. Any iprodione formed at this pH was unstable, such that at all temperatures the terminal product formed was RP 30228. The quantity of iprodione formed is temperature dependent with up to 34% at 13.5°C, 30% at 25°C and 28% at 40°C. The rate of transformation of iprodione was also temperature dependent being more rapid at higher temperature.

Table 7.2.1.1-12: Distribution of ^{14}C-RP 35606 and formation of hydrolysis products during incubation in buffer solution at pH 7 and various temperatures. Results are in % TAR

DAT	RP 35606	Iprodione	RP 30228	RP 37176
pH 7 / 13.5°C				
0	100.0	n.d.	n.d.	
1	76.7	16.9	7.2	
2	63.1	21.7	15.7	
5	34.8	34.2	28.2	
9	28.5	31.8	37.3	
13	25.9	28.1	45.3	
33	15.5	22.1	61.6	
50	13.9	n.d.	82.1	
pH 7 / 25°C*				
0	100.0	n.d.	n.d.	n.d.
1	51.4	32.5	12.0	n.d.
2	32.4	30.8	34.6	1.3
5	15.0	27.0	54.7	n.d.
9	9.7	11.4	77.2	n.d.
13	6.4	7.0	79.4	n.d.
23	3.0	1.3	92.6	n.d.
33	n.d.	n.d.	97.3	n.d.
50	n.d.	n.d.	96.5	n.d.
pH 7 / 40°C				
0	100.0	n.d.	n.d.	n.d.
1	24.2	27.5	43.5	n.d.
5	n.d.	4.3	94.0	n.d.
12	n.d.	4.9	85.8	4.3
22	n.d.	n.d.	99.7	n.d.
29	n.d.	n.d.	97.2	n.d.

* values are average of duplicate sampling
n.d. = not detected

The hydrolysis of RP 35606 at pH 8 proceeded more slowly than at pH 4 or 7, with the formation of both iprodione and RP 30228. Three other compounds were observed: RP 36233, RP 37176 together with 3,5-dichloroaniline.

The maximum quantity of iprodione detected was lower than at pH 7 and did not exceed 10%. This is consistent with the more rapid transformation of iprodione under alkaline conditions, resulting in the formation of RP 30228.

At this pH RP 30228 further degrades with the formation of RP 37176, RP 36233, and 3,5-dichloroaniline at 40°C. The formation rate was up to 20%, 7.0%, and 16.5% at 40°C, respectively.

Table 7.2.1.1-13: Distribution of ^{14}C-RP 35606 and formation of hydrolysis products during incubation in buffer solution at pH 8 and various temperatures. Results are in% TAR

DAT	RP 35606	Iprodione	RP 30228	RP 36233	RP 37176	3,5-dichloroaniline
pH 8 / 13.5°C						
0	100.0	n.d.	n.d.			
1	84.7	6.4	5.5			
2	76.7	6.9	14.1			
5	55.7	5.2	33.5			
9	47.6	6.7	48.7			
13	42.6	n.d.	51.9			
33	21.0	n.d.	73.7			
50	13.8	n.d.	83.5			
pH 8 / 25°C*						
0	100.0	n.d.	n.d.	n.d.	n.d.	
1	67.8	9.0	19.6	n.d.	n.d.	
2	51.8	5.3	38.9	n.d.	n.d.	
5	28.3	3.9	61.9	n.d.	n.d.	
9	23.5	n.d.	70.1	n.d.	n.d.	
13	17.4	n.d.	76.6	n.d.	n.d.	
23	20.2	n.d.	75.7	n.d.	n.d.	
33	21.3	n.d.	70.6	n.d.	3.6	
50	17.2	n.d.	64.5	n.d.	7.2	
pH 8 / 40°C						
0	100.0	n.d.	n.d.	n.d.	n.d.	n.d.
1	37.3	3.0	53.8	n.d.	n.d.	n.d.
5	23.8	n.d.	67.4	n.d.	6.2	n.d.
12	19.0	n.d.	55.4	0.9	5.1	16.0
22	15.8	n.d.	46.9	4.5	12.8	16.5
29	9.4	1.7	40.0	7.0	20.8	12.8**

* values are average of duplicate sampling
** the low amount may be due to evaporation of 3,5-dichloroaniline during analysis
n.d. = not detected

The hydrolysis of RP 35606 at pH 9 proceeded the slowest of all the pH values. At this pH iprodione was not detected although its role as a transitory intermediate was evident by the appearance of the hydrolysis product RP 37176.

The main degradation product formed at this pH level was RP 36233, which reached a maximum of 52% at 40°C. The product RP 37176 was also formed and reached a maximum of 21.4% at 40°C together with 3,5-dichloroaniline, which can be formed from both RP 36233 and RP 37176 and reached a maximum of about 22%.

The transformation product RP 30228 was rapidly formed; however, it was also rapidly degraded at this pH level, particularly at the higher temperatures.

Table 7.2.1.1-14: Distribution of ^{14}C-RP 35606 and formation of hydrolysis products during incubation in buffer solution at pH 9 and various temperatures. Results are in % TAR

DAT	RP 35606	Iprodione	RP 30228	RP 36233	RP 37176	3,5-dichloroaniline
pH 9 / 13.5°C						
0	100.0	n.d.	n.d.	n.d.		
1	92.0	n.d.	5.8	n.d.		
2	83.5	n.d.	15.3	n.d.		
5	72.3	n.d.	24.8	n.d.		
9	77.1	n.d.	19.8	n.d.		
13	76.3	n.d.	23.7	n.d.		
33	72.8	n.d.	18.1	7.8		
50	62.1	n.d.	22.5	6.9		
pH 9 / 25°C						
0	100.0	n.d.	n.d.	n.d.	n.d.	n.d.
1	79.7	n.d.	17.5	n.d.	n.d.	n.d.
2	75.7	n.d.	22.9	1.1	n.d.	n.d.
5	69.8	n.d.	22.2	5.9	n.d.	n.d.
9	71.0	n.d.	16.8	7.2	3.0	n.d.
13	63.9	n.d.	22.5	11.5	3.2	n.d.
23	51.8	n.d.	16.3	17.2	7.5	5.9
33	46.3	n.d.	12.0	25.4	8.3	5.6
50	35.1	n.d.	8.7	33.0	10.8	6.6
pH 9 / 40°C						
0	100.0	n.d.	n.d.	n.d.	n.d.	n.d.
1	81.9	n.d.	19.7	n.d.	n.d.	n.d.
5	57.7	n.d.	12.5	15.9	7.8	6.7
12	23.6	n.d.	7.6	30.5	17.3	17.6
22	6.8	n.d.	1.7	48.8	21.4	14.9
29	3.6	n.d.	n.d.	52.3	12.9	22.1

* values are average of duplicate sampling
n.d. = not detected

The rate of degradation of RP 35606 was highly pH dependent with half-life values ranging from 0.5 hours to greater than 50 days. A summary of RP 35606 degradation rates at various pH levels is given in Table 7.2.1.1-15.

Table 7.2.1.1-15: RP 35606 degradation rate in buffer solution at various pH range

Incubation time [days]	Incubation temperature [°C]	pH	DT_{50} [days]
50	13.5	4	0.02*
		7	3.0
		8	8.0
		9	>50
50	25	4	0.02*
		7	1.1
		8	2.1
		9	27.9
29	40	4	0.02*
		7	0.7**
		8	0.6
		9	5.8

* determined experimentally to be approximately 0.5 hours at room temperature
** extrapolated

RP 30228

The hydrolysis of [^{14}C]-phenyl ring labelled RP 30228 has been investigated in aqueous solution at pH 4, 7, 8 and 9. The study was conducted at 50 ± 0.3°C. The distribution of radioactivity during hydrolysis of RP 30228 at various pH levels is given in Table 7.1.1.3-10.

For the hydrolysis of [^{14}C]-phenyl ring labelled RP 30228, the total recovery of radioactivity was in the range from 90.2% to 99.3% of initially applied radioactivity.

At pH 4 and pH 7 RP 30228 was not hydrolysed at 50°C over the 5 day incubation period. At pH 8 and pH 9 RP 30228 was rapidly hydrolysed being more rapid at pH 9.

Table 7.2.1.1-16: Distribution of ^{14}C-RP 30228 and formation of hydrolysis products during incubation in buffer solution at various pH levels. Results are mean of duplicate samples in % TAR

DAT	RP 30228	Iprodione	RP 35606	RP 36233	RP 37176	3,5-dichloroaniline
pH 4 / 50°C						
0	100.0	n.d.	n.d.	n.d.	n.d.	n.d.
1	90.6	n.d.	n.d.	n.d.	n.d.	n.d.
5	98.9	n.d.	n.d.	n.d.	n.d.	n.d.
pH 7 / 50°C						
0	100.0	n.d.	n.d.	n.d.	n.d.	n.d.
1	90.2	n.d.	n.d.	n.d.	n.d.	n.d.
5	96.8	n.d.	n.d.	n.d.	n.d.	n.d.
pH 8 / 50°C						
0	100.0	n.d.	n.d.	n.d.	n.d.	n.d.
1	57.9	2.5	26.0	n.d.	3.8	6.7
5	31.6	n.d.	12.7	7.7	23.1	24.1
pH 9 / 50°C						
0	74.7	n.d.	26.1	n.d.	n.d.	n.d.
1	10.7	n.d.	47.5	19.8	5.7	13.2
5	n.d.	n.d.	n.d.	47.1	13.6	38.3

n.d. = not detected

At pH 8 and pH 9 RP 30228 was hydrolysed to RP 35606 which was then further degraded. The rate of formation and degradation of RP 35606 being more rapid at pH 9. Two other degradation products, RP 36233 and RP 37176, were also formed followed by further degradation to 3,5-dichloroaniline.

RP 36233 was formed by direct hydrolysis by opening the dioxoimidazolidine ring to RP 36233 which was then degraded to 3,5-dichloroaniline. The second pathway was by dioxoimidazolidine ring opening to RP 35606. RP 35606 was then further transformed to iprodione by re-cyclisation and iprodione was so unstable under these alkaline conditions that it rapidly degraded to RP 37176 that it was not possible to observe it in this study. The RP 37176 was then further degraded to 3,5-dichloroaniline.

The half-life value of RP 30228 at pH 8 was 1.8 days and at pH 9 the corresponding value was 0.3 days.

III. CONCLUSION

The results showed consistent pH dependence for the interconversion between iprodione, the ring opened intermediate hydrolysis product RP 35606 and the transposition metabolite RP 30228. At low pH iprodione is more stable than at high pH levels. Thus, if the pathway commences with iprodione under acidic conditions it will steadily proceed to RP 30228, under alkaline conditions this reaction proceeds more rapidly.

Commencing with the ring opened hydrolysis product RP 35606 the equilibrium at pH 4 lies towards iprodione whereas at pH 7, 8 and 9 it lies towards RP 30228. At pH 4 iprodione is stable so no further degradation takes place and at pH 7 RP 30228 is stable. Thus, only at pH 8 and 9 does further degradation at both ends of the equilibrium take place.

Commencing with RP 30228 the transposition compound is stable at pH 4 and 7 and no hydrolysis or degradation takes place.

The hydrolysis of iprodione and its hydrolysis and transposition products RP 35606 and RP 30228 was investigated at a wide range of pH levels and temperatures.

There was a pH dependent equilibrium between the three compounds that was relatively complex, with degradation taking place, depending upon the pH at both ends of the equilibrium.

There was no evidence for the presence of iprodione being formed from RP 30228 in these studies primarily for two reasons; that under acidic and neutral conditions RP 30228 is stable and under alkaline conditions iprodione is so much more rapidly degraded in comparison to its rate of formation.

CA 7.2.1.2 Direct photochemical degradation

A study on direct photochemical degradation is required for compounds with a molar (decadic) absorption coefficient (ε) > 10 L × mol –1 × cm –1 at a wavelength (λ) ≥ 295 nm determined with purified active substance. Iprodione does not meet that trigger. Nevertheless a direct photolysis study had been performed and **presented in the original Annex II Dossier (1995),** and will be briefly summarised here.

Report: Adrian, P. & Robles, J.M. (1991): ^{14}C-iprodione: Aqueous photolysis (Study 90-22).
Report AG/CRLD/AN no 9115524 of April 19, 1991.
R014564
GLP: Yes

A study investigating the photolytic breakdown of ^{14}C-phenyl iprodione in sterile, buffered aqueous solution has been carried out according to the Guidelines given by the U.S. E.P.A., subdivision N, section 161-2.
Irradiation was performed at 25°C ± 1°C and at a pH value of 5. After autoclaving, 99 mL of buffer solution was placed into glass photolysis vessels with quartz covers before treating with an acetonitrile solution of a mixture of iprodione (analytical standard) and ^{14}C phenyl iprodione, to give a final test concentration of *ca*. 5 mg/L. The concentration of the co-solvent (acetonitrile) did not exceed 1% (v/v). The photolysis samples were continuously exposed to simulated sunlight from an Original Hanau Suntest accelerated exposure machine.

Duplicate photolysis samples were analysed for parent compound and degradation products by TLC and HPLC after the equivalent of approximately t=0, 4, 9, 15, 33 days equivalent to Florida summer sunlight. Dark controls maintained at 25° ± 1°C were analysed the same way after 15 and 33 days irradiation time.

Results: Iprodione was not readily degraded in pH 5 buffer solutions and the estimation of the half-life using the above conditions was about 67 days compared to summer sunlight in Florida. No reaction products produced during the photolysis were found at a level greater than 10% of the applied radioactivity.

Analyses of the reaction products showed the presence of hydrolysis products (RP 30228, RP 32490), a hydroxylated product (RP 37677), a dechlorinated compound (RP 25331) and three dichloro isomers of iprodione (one of these was identified as RP 40837 and was considered as the predominant photolysis product). Some other polar photolytic products were not identified, each of them accounting for less than 7% of the applied radioactivity.

Table 7.2.1.2-1: Compounds, as % of starting material, with time

	0 days	4 days	9 days	15 days	33 days
Iprodione	97.3	88.6	89.0	68.4	67.8
RP 30228	0	0	1.0	2.0	1.9
RP 40837	0	2.2	0.6	1.0	1.3
Others	1.8	5.8	1.0	2.7	2.6

The presence of photosensitiser (acetone) did not change the nature of the identified products but accelerates the rate of photodegradation: half-life was estimated at 22 days equivalent exposure time compared to summer sunlight in Florida.

Report: Robles, M. & Maestracci, M. (1992): Iprodione: UV-Visible spectrum (Study no 92-01).
Report Rhône-Poulenc AG/CRLD/AN/9215288 of February 24, 1992.
R014573
GLP: Yes

A study was conducted to show it was not necessary to estimate the quantum yield of iprodione because no phototransformation occurs, and to prove in a GLP study that iprodione does not absorb between 295 and 650 nm. UV-visible characteristics of iprodione (purity 99.9%) in acetonitrile and in aqueous solutions were determined according to OECD guideline no 101.

Results: In deionised and purified water an absorption maximum was observed at 204.5 nm with an molar extinction coefficient of around 43000 (1 x mol^{-1} x cm^{-1}).
A similar figure was obtained for acetonitrile at 208 nm but that at 295 nm, in this solvent, was found to be less than 10 (1 x mol^{-1} x cm^{-1}). No significant modification of the spectrum was observed in acidic medium, and in deionised and purified water as well as in acetonitrile.

Table 7.2.1.2-2: Extinction coefficients in different solutions

Solution	Wavelength [nm]	Extinction coefficient E [l/mol cm]
HCl (0.1N)/acetonitrile (99:1)	206	43233
Water/acetonitrile (99:1)	204.5	44333
Acetonitrile	208	43362
Acetonitrile	295	2.07

CA 7.2.1.3 Indirect photochemical degradation

Study not required.

CA 7.2.2 Route and rate of biological degradation in aquatic systems

CA 7.2.2.1 "Ready biodegradability"

Studies presented in the original Annex II Dossier (1995):

Report: Muttzall, P.I. & Hansveit, A.O. (1989): Biodegradability of iprodione according to OECD guideline 301B (Modified Sturm-test).
Report no R 88/427 of January 16, 1989.
C022010
GLP: Yes

A modified Sturm-test has been conducted to assess the ready biodegradability of ^{14}C labelled iprodione. Results, after the 28 day test duration, showed that iprodione was not mineralised to carbon dioxide (only about 0.1% of the initial radioactivity was detected as radioactive CO_2) and therefore was considered as not readily biodegradable.

CA 7.2.2.2 Aerobic mineralisation in surface water

Not yet peer-reviewed studies

Report:	CA 7.2.2.2/1 Riefer P., 2014a 14C-BAS 610 F (Iprodione): Aerobic mineralisation in surface water - Simulation biodegradation 2013/1311349
Guidelines:	OECD 309 (April 2004)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The aquatic mineralisation of [phenyl-U-^{14}C]-iprodione was investigated in a pelagic test system with natural surface water containing small amounts of suspended sediment. Natural pond water (250 mL) test systems containing approx. 0.01 g/L dry weight equivalent of pond sediment were treated at two different test concentrations, 10 µg/L and 100 µg/L. Test vessels were incubated in the dark under aerobic conditions at a temperature of 20°C ± 2°C (except for 6 days, 20°C ± 3°C). Microbial activity of the test system was proven by the degradation of [7-^{14}C]-benzoic acid.

Duplicate samples were taken immediately after incubation (0 h) and at 1 h, 3 h, 6 h, 24 h, 48 h, 7 d, 14 d, 29 d and 46 d after treatment.

Aliquots of each sample were extracted with ethyl acetate and dichloromethane. The amount and nature of radioactivity in the water samples was determined by LSC and in organic extracts in addition by HPLC-UV and radio detection. Volatiles were trapped in appropriate trapping solutions and also analysed by LSC.

The material balance for the pelagic test samples ranged from 93% to 101% of the initially applied amount.

Iprodione was rapidly transformed to its two major transformation products. At the end of the incubation period (46 d), 11% to 19% AR could be associated with iprodione, whereas 57% to 59% AR was recovered as Reg.No. 5079647 (RP 30228) and 6% to 8% AR as Reg.No. 5079626 (RP 35606), with maximum occurrences of 65% AR RP 30228, day 14) and 30% (RP 35606, 6 h). In addition, minor amounts of Reg.No. 85831 (RP 32596) with a maximum occurrence of 2% AR, Reg.No. 5079632 (RP 36233) of 2% AR, Reg.No. 5079612 (RP 37176) of 3% AR and Reg.No. 5079628 (RP 32490) of 6% AR at day 46 were found.

Overall, the degradation of iprodione was characterised by a low mineralisation rate. The amount of radioactivity in the volatile traps never exceeded 1.0% AR within 46 days.

The degradation parameters for iprodione were calculated by FOMC kinetics, ranging from 0.23 to 0.27 days ($DegT_{50}$) and from 14.4 to 15.7 days ($DegT_{90}$). For the transformation products Reg.No. 5079626 (RP 35606) and Reg.No. 5079647 (RP 30228), $DegT_{50}$ values of 0.24 to 0.57 days and of 1.18 to 3.60 days were calculated, respectively.

The equal transformation product pattern of the viable test samples and the sterile samples as well as the low amount of mineralisation indicate that transformation is based predominantly on abiotic hydrolysis and only by a negligible percentage on microbial degradation.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material BAS 610 F
CAS #: 36734-19-7
Molar mass: 330.17 g mol^{-1} (non-labelled)
Solubility in water: pH 3 = 13 mg L^{-1} (20°C); pH 7 = 12.2 mg L^{-1} (20°C), pH 9 = unstable

Labelled test item
Label: [phenyl-U-^{14}C]-labelled
Specific radioactivity: 61.8 MBq g^{-1}
Radiochemical purity: 99.1%; 93% before treatment (HPLC)

Unlabelled test item
Chemical purity: 97.8%

B. STUDY DESIGN

1. Test system

Water and surface sediment were freshly collected from a pond “Reinheimer Teiche”, Reinheim, Germany (N49°51.130’ E08°50.998’). The water was collected about 4 m from the bank and about 10 cm below the water surface. The sediment was collected at the same place as the water (approx. 4 m from the bank). The upper sediment layer (approx. 5 mm) was removed prior to storage. Samples were stored for 7 days at approx. 4°C under dark conditions until use. The sediment was stored with a water layer above the sediment. Prior to use the sediment was sieved (2 mm). The river water and sediment characteristics are summarised in Table 7.2.2.2-1.

Table 7.2.2.2-1: Pond water and sediment characteristics

Name:		Reinheimer Teiche
Origin:		64354 Rheinheim, Germany N49°51.130' E08°50.998'
Sampling date:		08 November 2013
Water parameters measured at field sampling		
Temperature	[°C]	12.2
pH (water)		8.2
Oxygen concentration	[%]	137.4
Redox potential (E_h) *	[mV]	185
Sampling depth	[cm]	0 – 10
Colour		Yellowish
Turbidity/Visibility		Slightly turbid
Sediment parameters measured at field sampling		
pH (water)		6.8
Oxygen concentration	[mg/L]	0.05
Redox potential (E_h) *	[mV]	-165
Water parameters measured post-handling		
TOC	[mg/L]	3.9
DOC	[mg/L]	3.2
Nitrate	[mg/L]	5.75
Nitrite	[mg/L]	0.033
Ammonium	[mg/L]	0.226
Orthophosphate	[mg/L]	0.005
N total	[mg/L]	0.76
P total	[mg/L]	0.038

2. Experimental conditions

The sediment was passed through a 2 mm sieve, and the water was filtered through a 0.125 mm sieve. The flasks (250 mL glass bottles) were filled with about 250 mL of water and a very small amount of sediment solids was added to the test water (0.01 g/L dry weight) in order to provide a minimum of mineral and nutrient source for the microbial population. Since only 2.5 mg were added to the test vessel, the test system can still be considered pelagic.

The nominal application rates were achieved by pipetting into the upper water of the test vessels. For the low concentration (10 µg/L) 155 µL of a 16.19 mg/L application solution was used. For the high concentration (100 µg/L) 158 µg/L of a stock solution of 158.59 mg/L was applied.

For each sampling time point, one sterile sample was prepared by autoclaving the test vessels (20 min, 121°C).

For viability control, individual samples were prepared at the beginning, in the middle and at the end of the iprodione test phase. Test vessels containing 250 mL surface water were treated with 115 µL of the ^{14}C-benzoic acid stock solution (with a concentration of 105.59 mg/L) in order to achieve a nominal application rate of 50 µg/L.

After application of the test item, each test vessel was connected to the air stream leading to a trapping system of five gas washing bottles containing different trapping solutions for the ^{14}C-volatiles to be expected: 1) 15 mL pure water, 2) 15 mL ethylene glycol, 3) 15 mL 0.05 M H_2SO_4, 4) 15 mL 2 M NaOH and 5) 15 mL 2 M NaOH. The test vessels for the pelagic test were placed on multiplate magnetic stirrers.

At each sampling an aliquot of 1.6 mL was taken and the test vessels were re-connected to the flow-through system.

Test vessels were incubated in the dark under aerobic conditions at a temperature of 20°C ± 2°C (except for 6 days, 20°C ± 3°C).

3. Sampling

Duplicate samples were taken immediately after incubation (0 h) and at 1 h, 3 h, 6 h, 24 h, 48 h, 7 d, 14 d, 29 d and 46 d after treatment.

Individual viability tests were carried out at the beginning, middle (main test: 12 DAT) and end (main test: 40 DAT) of the entire iprodione test phase. Aliquots of the samples (batch approach) treated with ^{14}C-benzoic acid were taken after 0 h, 1 d, 3 d and 6 d.

4. Description of analytical procedures

Immediately after sampling, aliquots were taken to measure the radioactivity in the water by LSC (1 mL) and to extract the appropriate aliquot (low conc: approx. 120 mL, high conc: approx. 50 mL) to stop potential further transformation of the test item and possible transformation products. HPLC analysis of the latter aliquots was carried out after concentration.

Two extraction steps of the aliquots were carried out. In the first extraction step the aliquot of the surface water was extracted three times, twice with ethyl acetate and once with dichloromethane. In the second extraction step, the same aliquot of the test water was extracted another three times including a shift of the pH to change the polarity of remaining compounds and thus increase their extractability. The pH was adjusted by adding dropwise 1 N HCl to the test water. The aliquot was first extracted at pH 5 with ethyl acetate, then at pH 2-3 with ethyl acetate and third at pH 2-3 with dichloromethane.

Samples were concentrated after extraction by evaporation until dryness. The samples were re-dissolved in acetonitrile/pure water 80/20 v/v.

For viability control two aliquots were taken per test vessel, one for LSC measurement (1 mL) and one for HPLC analysis (0.6 mL which was diluted 1:1 v/v with acetonitrile).

Aliquots of volatile traps were measured by LSC.

Measurements of pH, oxygen content, conductivity and redox potential were performed with respective electrodes.

Structural characterisation was accomplished by using a HPLC Sys1 for the test item and transformation product characterisation (Reg.No. 5079626 and 5079647). The HPLC Sys2 as secondary method of analysis was conducted in order to confirm the results of the HPLC method Sys1.

Since two unknown transformation products were detected during first analysis with Sys1 and Sys2, additional reference items were subjected to HPLC Sys1 and a further HPLC Sys3 in order to check for potential matching retention times. Selected samples (100 µg/L, test samples 24 h and 46 d after treatment, sterile samples 48 h and 46 d after treatment) were re-injected under the same HPLC conditions to facilitate peak assignment.

In terms of low concentration LOD was 0.3% AR and LOQ 1.0% AR. In case of high concentration LOD was 0.1% AR and LOQ 0.2% AR.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The mass balance for the pelagic test ranged from 93% to 101% AR. In the sterile vessels, the mass balance ranged from 95% to 101% AR. Volatiles were <1% in all test systems and during the whole incubation period.

Table 7.2.2.2-2: Material balance and distribution of radioactivity (LSC) after application of ^{14}C-iprodione [%AR]

DAT	Aqueous phase [1] (mean) [%AR]	Volatiles (mean) [%AR]	Recovery (mean) [%AR]
Test samples – low concentration 10 µg/L			
0	100	n.a.	100
0.04	99	<LOQ	99
0.125	101	<LOQ	101
0.25	99	<LOQ	99
1	100	<LOQ	100
2	101	<LOQ	101
7	98	0.45	99
14	99	0.12	100
29	99	0.12	99
46	100	0.42	100
Test samples – high concentration 100 µg/L			
0	100	n.a.	100
0.04	97	<LOQ	97
0.125	99	<LOQ	99
0.25	98	<LOQ	98
1	97	0.04	97
2	97	0.07	97
7	96	0.20	97
14	97	0.16	97
29	94	0.35	94
46	93	0.62	93
Sterile samples – 100 µg/L			
0	101	n.a.	101
0.04	97	<LOQ	97
0.125	100	<LOQ	100
0.25	99	<LOQ	99
1	100	<LOQ	100
2	100	<LOQ	100
7	99	0.06	99
14	94	0.14	95
29	99	0.17	99
46	101	0.14	101

n.a. - not applicable

[1] analysis of the test water prior to extraction

B. FINDINGS

Physico-chemical parameters of the test systems

The oxygen concentrations measured in the surface water of untreated control samples proved aerobic conditions during the incubation period. pH values of surface water during the incubation showed stability of the system.

Degradation of the reference test item

HPLC analysis showed that [7-^{14}C]-benzoic acid was completely degraded after 3 days of incubation in course of each viability test. The result showed that the quality (i.e. microbial activity) of the natural water sample was sufficient for the test.

Distribution of radioactivity

The actual applied amounts of iprodione per test vessel containing approx. 250 mL of water were 2.5 µg (low concentration) and 25.7 µg (high concentration).
In general, no significant differences in iprodione behaviour were found between both concentrations. Therefore, the results expressed in % of applied radioactivity (AR) mentioned in the text include those of both concentrations.

The material balance and the distribution of radioactivity in the pelagic test are shown in Table 7.2.2.2-3. The material balance for the pelagic test ranged from 93% to 101% AR. In the sterile vessels, the material balance ranged from 95% to 101% AR.
The amount of radioactivity in the water was found to be very stable. At the end of the study (46 DAT) it ranged from 93% to 100% AR. For all pelagic test samples and sampling time points the radioactivity in the volatile traps never exceeded 1.0% AR indicating a low rate of mineralisation.

Characterisation and identification of residues in extracts

In the pelagic test, iprodione was found to be rapidly transformed mainly to Reg.No. 5079626. After 1 day, only 29% to 33% AR could be recovered as unchanged parent for the different concentrations. At 46 DAT 11% to 19% AR could be associated with iprodione, whereas 57% to 59% AR was recovered as Reg.No. 5079647 (RP 30228) and 6% to 8% AR as Reg.No. 507926 (RP 35606).

After extraction of the test water and analysis of the extracts with Sys 1 two transformation products “Unknown 1” and “Unknown 2” could be found and accounted for 4% to 5% AR and 9% to 12% AR, respectively, after 46 days of incubation. Further characterisation of both unknown transformation products was carried out by using additional reference items and analysis methods Sys 1 and Sys 3. With Sys 3 “Unknown 1” could be assigned to Reg.No 85831 (RP 32596) with a maximum occurrence of 2% AR and “Unknown 2” could be separated in three known transformation products Reg.No. 5079632 (RP 36233) with a maximum occurrence of 2% AR, Reg.No. 5079612 (RP 37176) of 3% AR and Reg.No. 5079628 (RP 32490) of 6% AR at day 46.

In the sterile samples, 12% AR could be recovered as iprodione, 62% AR as Reg.No. 5079647 RP 30228) and 8% as Reg.No. 5079626 (RP 35606) after 46 days of incubation.
In addition (after re-analysis of the extracts with Sys 1 and Sys 3) minor amounts of Reg.No. 85831 (dichloroaniline) with a maximum occurrence of 1% AR, Reg.No. 5079632 (RP 36233) of 1% AR and Reg.No. 5079628 (RP 32490) of 3% AR were found at day 46. The results of the sterile samples are almost equal to the viable test samples.

The distribution of radioactivity during natural aqueous surface mineralisation of iprodione at two different test concentrations is shown in Table 7.2.2.2-3.

Table 7.2.2.2-3: Recovery and distribution of radioactivity during natural aqueous surface mineralisation of ^{14}C-iprodione - HPLC results; mean of two replicates [%AR]

DAT	RP 30228 [%AR]	Iprodione [%AR]	RP 35606 [%AR]	Unknown 1 [%AR]	Unknown 2 [%AR]	Recovery [%AR]
Low test concentration						
0	4	89	3	2	4	101
0	4	90	3	6	4	107
0.04	6	75	7	1	3	93
0.04	9	78	5	2	4	98
0.125	10	57	21	1	4	93
0.125	15	63	14	3	4	100
0.25	15	47	24	2	5	93
0.25	12	49	26	3	6	96
1	41	33	17	5	3	99
1	39	31	18	3	5	96
2	49	20	21	4	6	100
2	51	21	23	3	4	103
7	63	16	11	3	2	94
7	62	15	14	3	2	96
14	63	15	11	3	4	96
14	64	13	11	4	4	96
29	58	15	12	4	8	96
29	59	15	13	3	7	97
46	58	18	7	5	12	98
46	59	19	6	4	9	96
High test concentration						
0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
0	2	84	5	2	4	97
0.04	4	74	12	2	4	97
0.04	3	69	8	1	4	85
0.125	8	51	22	1	5	87
0.125	9	49	25	1	5	89
0.25	22	43	14	1	4	84
0.25	18	37	30	2	4	91
1	35	29	28	2	4*	98
1	37	32	26	2	4	101
2	48	19	20	3	4	94
2	46	16	22	3	3	90
7	61	14	10	3	4	92
7	61	16	10	3	4	94
14	64	12	10	3	5	94
14	65	12	10	2	5	94
29	56	14	10	2	10	92
29	56	14	11	3	9	93
46	*12*	*7*	*39*	*22*	*13*	*94*
46	57	11	8	4	11*	91

n.a. - not applicable (The test item was not applied to sample P21 but twice to sample P22)

in italic: outlier, will not be used for result evaluation

* further separated in different HPLC system and compounds identified by comparison of retention with reference compounds

For further characterisation selected samples (100 µg/L test samples incubated for 1 d and 46 d as well as sterile samples incubated for 2 d and 46 d) were re-analysed with additional reference items using methods Sys1 and Sys3.

Table 7.2.2.2-4: Recovery and distribution of radioactivity of selected samples (high test concentration) - HPLC results – Sys 1; mean of two replicates and sum of extraction steps [%AR]

	Test samples [%AR]		Sterile samples [%AR]	
Reg. No.	**1 d**	**46 d**	**2 d**	**46 d**
5079647 (RP 30228)	45	56	53	61
Iprodione	31	11	28	12
5079626 + 5079618 (RP 35606 + RP 36221)	18	9	11	10
85831 (Dichloroaniline)	3	4	2	4
5079632 + 5079612 + 5079628 (RP 36233 + RP 37176 + RP 32490)	4	10	5	10
Recovery	101	91	99	97

Table 7.2.2.2-5: Recovery and distribution of radioactivity of selected samples (high test concentration) - HPLC results – Sys 3; mean of two replicates and sum of extraction steps [%AR]

	Test samples [%AR]		Sterile samples [%AR]	
Reg. No.	**1 d**	**46 d**	**2 d**	**46 d**
5079647 (RP 30228)	39	55	52	60
5079618 (RP 36221)	1	<LOD	n.d.	n.d.
5079626 (RP 35606)	30	13	14	15
Unknown	1	1	1	n.d.
Iprodione	27	8	20	17
85831 (dichloroaniline)	1	2	2	1
5079632 (RP 36233)	1	2	2	1
5079612 (RP 37176)	n.d.	3	3	n.d.
5079628 RP 32490)	2	6	5	3
Recovery	101	90	99	97

n.d. not detected

Degradation rates

Kinetic analysis and calculations of $DegT_{50}$ and $DegT_{90}$ values were performed following the recommendations of the FOCUS Kinetics workgroup on derivation of aquatic persistence endpoints [*FOCUS (2006)*]. The analysis was done by a non-linear regression method (Iteratively Reweighted Least Squares) using the software package KinGUI II.

The evaluation of the results for high and low test concentrations showed that degradation of iprodione in the water phase was best described by FOMC kinetics. Degradation of the transformation products RP 35606 and RP 30228 could be sufficiently described with SFO kinetics. The transformation of iprodione to RP 35606 and RP 35606 to RP 30228 and vice versa was adequately described when the pathway was modelled.

Best-fit endpoints are summarised in the table below.

Table 7.2.2.2-6: Best-fit endpoints for iprodione and its transformation products RP 35606 and RP 30228

Compound	Test concentration	Kinetic model	Best-fit endpoints	
			$DegT_{50}$ [d]	$DegT_{90}$ [d]
Iprodione	Low	FOMC	0.27	15.7
	High	FOMC	0.23	14.4
RP 35606	Low	SFO	0.24	0.79
	High	SFO	0.57	1.91
RP 30228	Low	SFO	1.18	3.93
	High	SFO	3.60	12.0

III. CONCLUSION

From the obtained results it can be concluded that iprodione is rapidly transformed in a pure water environment. Two major transformation products were found: Reg.No 5079647 (RP 30228) and Reg.No 5079626 (RP 35606) with maximum occurrences of 65% AR (day 14) and 30% AR (6 h), respectively. In addition only minor amounts of Reg.No. 85831 (RP 32596), Reg.No. 5079632 (RP 36233), Reg.No. 5079612 (RP 37176) and Reg.No. 5079628 (RP 32490, dichloroaniline) were found at day 46 with maximum occurrences of 2% AR, 2% AR, 3% AR and 6% AR, respectively.

The degradation parameters for iprodione were calculated, ranging from 0.23 to 0.27 days ($DegT_{50}$) and from 14.4 to 15.7 days ($DegT_{90}$). For the transformation products Reg.No. 5079626 (RP 35606) and Reg.No. 5079647 (RP 30228), $DegT_{50}$ values of 0.24 to 0.57 days and of 1.18 to 3.60 days were calculated, respectively.

The equal transformation product pattern of the viable test samples and the sterile samples as well as the low amount of mineralisation indicate that transformation is based predominantly on abiotic hydrolysis and only by a negligible percentage on microbial degradation.

CA 7.2.2.3 Water/sediment studies

Several studies were performed to address the behaviour of iprodione in water sediment systems. A study performed by Spare [*Spare (1991) – Aerobic aquatic metabolism of iprodione*] using paddy rice water and sediment was not considered relevant for the applied uses of iprodion due to extremely alkaline pH of the water. In a second report Spare [*Spare (1991) – C025174*] investigated the degradation of iprodione under anaerobic conditions and Purser [*Purser (1992) – 1992/1001484*] investigated the degradation of iprodione in two water/sediment systems.

Report: Spare, W.C. et al. (1990): Anaerobic aquatic metabolism of iprodione. Report Agrisearch Inc no R 1510 of December 18, 1990. C025174
GLP: Yes

The rate and pattern of iprodione metabolism under anaerobic aquatic conditions was studied for 365 days (subsequent to an initial one month pre-incubation period) in the dark at 25 ± 1°C and a pH between 6.4 -7.4, using a silt loam flooded with water, dosed with ^{14}C-iprodione at about 6 mg/L.

Results: During 12 months anaerobic incubation of iprodione, minimal production of volatile products was observed and the majority of the dose moved from the water to the sediment: The metabolite degradation pathway of iprodione was a loss of parent with production of RP 30228 followed by metabolic degradation of RP 30228 to polar products covalently bound to the sediment organic matter. The recoveries of radioactivity were up to six months but were only 67.5 and 89% at 9 and 12 months respectively.

The half-life of iprodione was found to be 33 days. The major degradate was the isomeric RP 30228 along with minor amounts of RP 32490, RP 36221 and RP 32596. Production of RP 30228 reached a maximum of approximately 60% of applied radioactivity at 30 days. From 30 to 170 days, RP 30228 rapidly declined to approximately 10% of dose and plateaued.
Very polar extractable and covalently bound products were produced as RP 30228 declined. Polar products accounted for 20 to 30% of dose and bound residue 20% of dose at 12 months.

Table 7.2.2.3-1: Distribution of radioactivity (% dose) in water and sediment

Day	Iprodione	RP 30228	RP 36221/ RP 32490	RP 32596 (3,5-DCA)	Origin
0	99.8	0	0	0	0.2
1	97.9	0.1	2.4	0	2.0
2	95.7	1.4	0.9	0	0.7
3	92.4	1.7	1.0	0	0.7
7	85.9	8.8	0.8	0	1.7
14	29.9	59.8	0.9	0	2.4
30	19.2	61.6	5.1	0	3.2
94	8.2	47.3	0.7	0.9	19.6
170	4.4	12.3	0.9	0.4	45.9
275	0.8	4.9	1.7	3.5	29.5
365	3.4	13.4	1.1	2.4	25.0

Conclusion: The half-life of iprodione was found to be 33 days in the whole system (water plus sediment).
A proposed degradation pathway is shown in Figure 7.2.2.3-1.

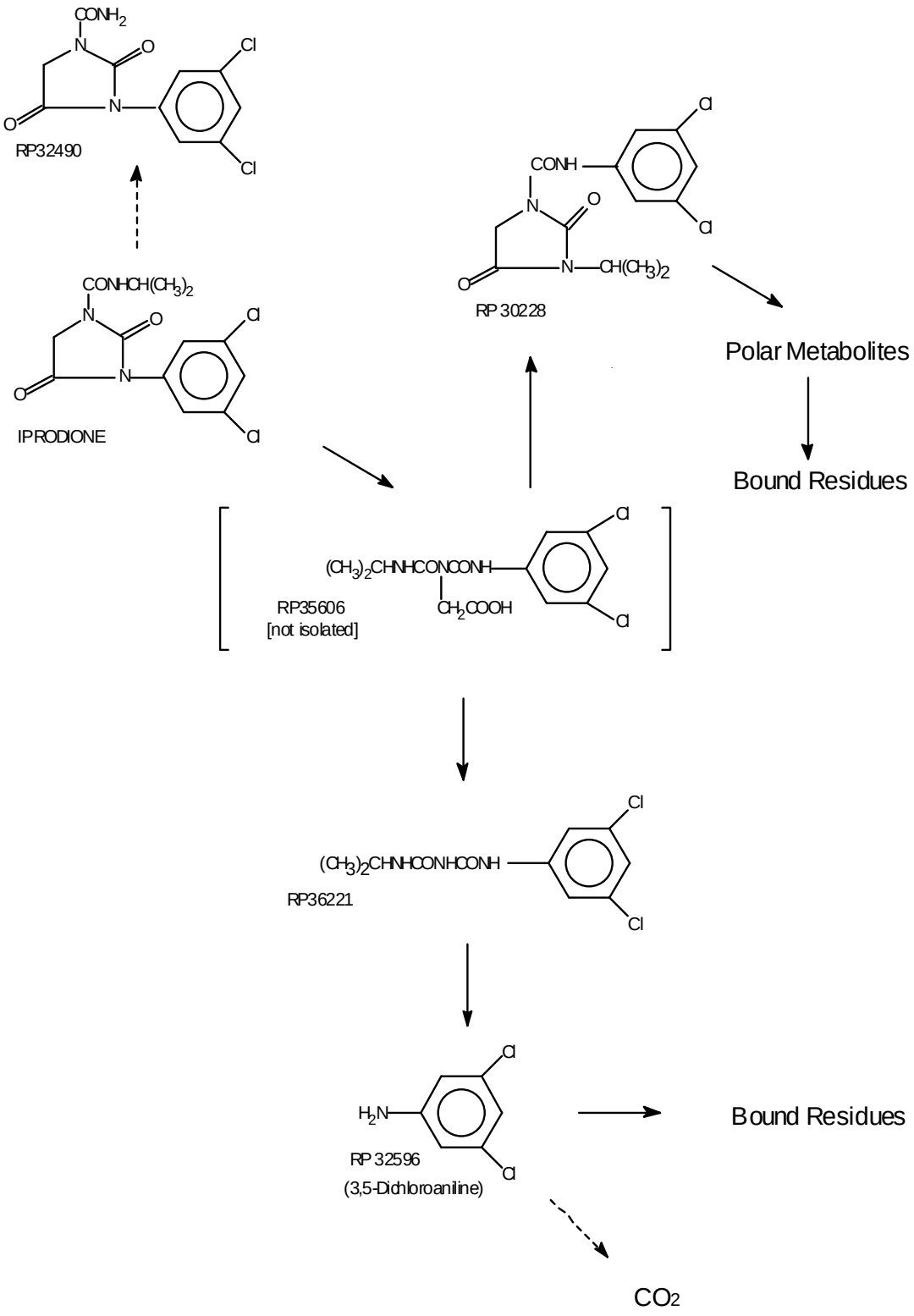


Figure 7.2.2.3-1: Proposed degradation pathway of iprodione under anaerobic aquatic conditions

Study presented in the original Annex II Dossier (1995):

Report: Purser, D. (1991): (^{14}C)-iprodione: Degradation and retention in water/sediment systems.
Report Hazleton UK no 7397 (study no 68/113) of December 8, 1992.
BASF DocID 1992/1001484, Report No. B003654

GLP: Yes

The degradation of (^{14}C)-phenyl labelled iprodione was studied in two aquatic systems (Mill Stream Pond and Iron Hatch pool run-off) maintained in the dark at 20 ± 2°C, over a 100 day period, following a single application to the water surface at a rate equivalent to 5 kg/ha (sediment was added to glass cylinders, ca. 4.5 cm diameter, to a depth of 2.5 cm and covered with associated water to a depth of 6 cm above the soil).

The systems were pre-incubated for 102 days prior to application. Samplings and analyses were performed at zero time and after 6, 24 and 48 hours and 7, 14, 30, 59 and 100 days after application. Moistened carbon dioxide-free air was drawn over the water surfaces and then through a sequence of traps to collect polar and non-polar organic volatiles and evolved carbon dioxide. The mean zero time and 100 day surface water pH values were 7.91 and 7.57 for Iron Hatch and 7.94 and 7.41 for Mill Stream Pond water/sediment units.

Table 7.2.2.3-2: Characteristics of sediment samples

Sediment characteristics	Source	
	Mill Stream Pond	Iron Hatch pool run-off
Particle size distribution (BBA) > 20 mm [%]	35	95
2mm - 20mm [%]	44	4
< 2mm [%]	21	1.0
Dry mass [%]	18	62.0
Organic carbon [%]	4.4	0.6
pH (H_20)	7.2	7.0
pH (KCl)	6.9	7.0
Total Nitrogen [g/kg]	3.1	0.8
Total Phosphorus [mg/kg]	26.6	5.8
Cation exchange capacity [me/100g]	75.9	12.5

Table 7.2.2.3-3: Characteristics of surface water samples

Surface water characteristics	Source	
	Mill Stream Pond	Iron Hatch pool run-off
Total Nitrogen [mg/L]	5.1	5.7
Total Phosphorus [mg/L]	158.1	163.6
Total Organic carbon [mg/L]	2.9	2.9
Water Hardness [mg/L as $CaCO_3$]	241.3	234.4

Results: The partition of radioactivity between the water and sediment phases of both systems was similar. Immediately after application, > 76% of the applied radioactivity was recovered in the surface water, then there was a transfer of radioactivity to the sediment which contained 77 to 81% after 100 days (surface water contained 3 to 9% of the applied radioactivity after 100 days). After an apparent lag period of 7 days or more, unextracted residues in the sediment increased rapidly to 13 -15% after 30 days incubation and decreased slowly thereafter to 5 to 10% at 100 days.

The iprodione isomer (RP 30228) and the ring-opened hydrolysis product (RP 35606) were the major components in all water samples and sediment extracts. Surface water from both systems contained the majority (69% to 73%) of the applied radioactivity as the hydrolysis product (RP 35606) at zero time. This compound declined to < 4% in each at 59 days after application. The isomeric RP 30228 reached a maximum of 10% in both systems within 24 hours and declined more slowly than the parent compound thereafter. Neither parent nor isomer was detected in surface water after 59 days incubation. Parent compound reached maxima of 6 and 8% within 24 hours, declining rapidly thereafter.

^{14}C-iprodione degraded rapidly: in both aquatic systems. The DT_{50} was < 6 hours. The DT_{90} of ^{14}C-iprodione in the Iron Hatch pool run-off system was 32 days and the Mill Stream Pond System was 10 days.

Table 7.2.2.3-4: Mean % of applied radioactivity determined as iprodione and its degradation products in surface water (Mill Stream Pond)

Sampling	Iprodione	RP 30228	RP 35606	Unknown 1	Total
0 hours*	1.7	7.8	69.3	nd	78.7
6 hours	2.1	5.2	56.7	nd	64.0
24 hours	7.5	9.6	50.1	nd	67.2
48 hours	6.8	9.4	38.2	nd	54.3
7 days	2.5	6.9	17.2	nd	26.6
14 days	0.8	2.2	6.5	0.1	9.5
30 days	0.4	0.5	3.3	nd	4.2
59 days	nc	nc	nc	nc	nc
100 days	nc	nc	nc	nc	nc

* nominal '0 h' sampling; does not include sample processing time
nd = not detected
nc = not characterised (low activity)

Table 7.2.2.3-5: Mean % of applied radioactivity determined as iprodione and its degradation products in acetonitrile extracts of sediment (Mill Stream Pond)

Sampling	Iprodione	RP 30228	RP 35606	Unknown 1	Total
0 hours*	7.9	6.3	nd	nd	14.2
6 hours	5.6	11.7	4.0	nd	21.3
24 hours	4.3	19.2	1.1	0.8	25.3
48 hours	4.3	25.1	1.7	0.9	32.0
7 days	15.0	45.3	3.5	nd	63.7
14 days	10.8	62.7	nd	nd	73.5
30 days	6.4	60.0	nd	1.6	68.0
59 days	6.3	67.0	nd	2.5	75.8
100 days	nd	79.2	nd	nd	79.2

* nominal '0 h' sampling; does not include sample processing time
nd = not detected
nc = not characterised (low activity)

Table 7.2.2.3-6: Mean % of applied radioactivity determined as iprodione and its degradation products in surface water (Iron Hatch)

Sampling	Iprodione	RP 30228	RP 35606	Unknown 1	Total
0 hours*	3.2	5.0	73.3	nd	81.5
6 hours	6.4	2.3	56.4	1.0	66.1
24 hours	4.7	10.3	62.1	nd	77.1
48 hours	4.3	9.1	43.8	nd	57.2
7 days	4.3	7.4	21.4	nd	33.1
14 days	1.4	3.1	7.6	0.6	12.6
30 days	0.2	3.6	0.7	nd	4.5
59 days	nc	nc	nc	nc	nc
100 days	nc	nc	nc	nc	nc

* nominal '0 h' sampling; does not include sample processing time
nd = not detected
nc = not characterised (low activity)

Table 7.2.2.3-7: Mean % of applied radioactivity determined as iprodione and its degradation products in acetonitrile extracts of sediment (Iron Hatch)

Sampling	Iprodione	RP 30228	RP 35606	Unknown 1	Total
0 hours*	9.5	1.2	nd	nd	10.6
6 hours	10.9	9.1	nd	nd	19.9
24 hours	11.7	7.2	nd	nd	18.9
48 hours	15.4	23.2	nd	nd	38.6
7 days	20.0	42.6	nd	nd	62.6
14 days	10.8	61.7	nd	nd	72.4
30 days	5.1	62.9	nd	0.7	68.7
59 days	9.1	70.2	nd	2.8	82.1
100 days	3.6	72.1	nd	nd	75.7

* nominal '0 h' sampling; does not include sample processing time
nd = not detected
nc = not characterised (low activity)

Conclusion: ^{14}C-iprodione degraded rapidly: in both aquatic systems. The DT_{50} was < 6 hours. The DT_{90} of ^{14}C-iprodione in the Iron Hatch pool run-off system was 32 days and the Mill Stream Pond System was 10 days.

The study on the degradation of iprodione in water/sediment systems performed by Purser [*Purser (1992) – 1992/1001484*] summarised above was evaluated in the Annex I inclusion process and is still considered valid. The proposed degradation pathway is presented in Figure 7.2.2.3-2. Due to changes in the guidelines, a new kinetic evaluation was performed, which is summarised below (CA 7.2.2.3/1).

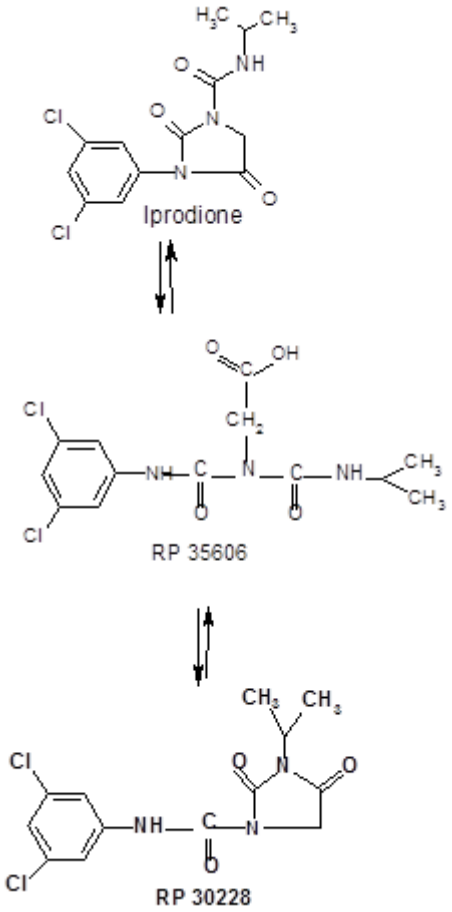


Figure 7.2.2.3-2: Proposed degradation pathway of iprodione in water/sediment systems

Not yet peer-reviewed study:

Report:	CA 7.2.2.3/1 Gurney A., 2008a Iprodione: Calculation of degradation rates in water-sediment systems - Kinetic analysis using KinGUI and ModelMaker 2008/1028646
Guidelines:	EEC 95/36 Annex II 9.2.3 Estimation of concentration in surface water, FOCUS Kinetics (2006)
GLP:	No, not subject to GLP regulations

Executive Summary

Kinetic analysis was performed on the data from two aerobic water-sediment systems that were incubated under laboratory conditions in the dark for a period of 100 days. The evaluation followed the guidance of the FOCUS workgroup on degradation kinetics, in order to derive degradation half-lives suitable for use in model calculations of predicted concentrations in surface water.

The isomer coded RP 30228 and a spontaneously formed intermediate hydrolysis product (RP 35606) of iprodione were observed as the major components in all samples. Therefore, for the purpose of kinetic analysis, the sum of residues of iprodione and the intermediate hydrolysis product RP 35606 was taken as total 'parent' iprodione, combined with the assumption that this total 'parent' residue degraded directly to the isomer RP 30228 as the primary surface water degradation product. In a first step, total water-sediment system degradation half-lives were fitted for iprodione and its metabolite RP 30228 using the software tool KinGUI and a simple one-compartment approach (Level I), according to single first-order (SFO) kinetics. The results of kinetic analysis for the two water-sediment systems showed that SFO fits were visually and statistically acceptable. Therefore, the calculated half-lives for total systems are considered appropriate as modelling endpoints to result in a geometric mean half-life of 6.9 days for the sum of iprodione and its hydrolysis product RP 35606. The major surface water metabolite RP 30228 was found to degrade with a geometric mean half-life of 63 days.

In a subsequent analysis, a ModelMaker compartmental model, with two individual compartments representing water and sediment, was used in an attempt to calculate separate half-lives for water and sediment degradation of total 'parent' iprodione (Level P-II). The results of these ModelMaker calculations were found to be statistically unreliable. On the basis of this analysis, it is recommended to parameterise simulation models using the total-system half-life of iprodione for the water-phase, and a conservative default half-life for the sediment-phase, when separate degradation rates are required for the water and sediment compartments.

I. MATERIAL AND METHODS

The kinetic evaluation of iprodione was conducted for one study with two aquatic systems, Iron Hatch and Mill Stream Pond [*Purser (1992) – 1992/1001484*].

In the study, the test item (^{14}C)-iprodione was applied to the two water-sediment systems at a rate equivalent to 5 kg ha^{-1}. Incubations were performed in glass cylinders containing sediment to a depth of 2.5 cm covered with associated water to a depth of 6 cm above the sediment, maintained in the dark at 20 ± 2°C. Iprodione is non-volatile (vapour pressure 5 x 10^{-7} Pa at 25 °C), so that disappearance from the total system can be attributed to degradation.

Kinetic evaluation was performed in order to derive modelling endpoints according to the guidelines 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 2.0, 434 pp*].

Kinetic modelling

For the purpose of kinetic analysis, the sum of residues of iprodione and the intermediate hydrolysis product RP 35606 was taken as total 'parent' iprodione, combined with the assumption that this total 'parent' residue degraded directly to the isomer RP 30228 as the primary surface water degradation product.

In a first step, total water-sediment system degradation half-lives were fitted for total 'parent' iprodione residues and the major surface water metabolite RP 30228 (i.e. treating water and sediment as a single compartment, Level I) using single first order kinetics. In a second step, two individual compartments representing water and sediment was used in an attempt to calculate separate half-lives for water and sediment degradation of total 'parent' iprodione residues (Level P-II).

At the first stage of the total system evaluation (Level I), single first-order (SFO) fits to the total 'parent' iprodione data in the total-system were performed. Having established that SFO fits for parent were statistically and visually acceptable, the metabolite was introduced to the conceptual model and fitted again by use of single first-order (SFO) kinetics with regard to the formation and decline data of RP 35606 (Level M-I based on metabolite formation and degradation). The second stage of Level I analysis itself was performed in two sub-steps. Initially, the parameters for the parent kinetic fit were fixed to the values already obtained, and the parameters for the metabolite were fitted. Residue of RP 30228 detected at time-zero was included in the fitting process and the parameter describing the initial mass of metabolite was optimised. Therefore three parameters were fitted in this initial sub-step: the initial mass of metabolite, the degradation rate of metabolite and the molar formation fraction. At the final stage of the fitting process of Level I, the parameter values determined for total 'parent' iprodione and metabolite RP 30228 in the previous steps were used as initial values, and they were then re-optimised simultaneously according to FOCUS guidance to determine the total-system half-lives for both total 'parent' and metabolite. The values of the molar formation fraction for RP 30228 in both water-sediment systems were found not to differ significantly from 1 in the previous sub-step. The value of the molar formation fraction was therefore fixed at 1 at this final stage of the Level I fitting procedure.

In a subsequent kinetic analysis (Level P-II), two individual compartments (water compartment and sediment compartment) were set up in an attempt to calculate separate half-lives for water and sediment degradation of total 'parent' iprodione. Parameters were fitted to the water and sediment residues in a single optimisation step.

Data handling

For kinetic analysis, residues of iprodione and RP 35606 were added to give the total 'parent' iprodione levels. The limit of detection (LOD) was assumed to be 0.4 %AR based on the lowest reported value of 0.41 %AR. Values reported as 'not detected' immediately following positive detections were set to ½ LOD, i.e. 0.2 %AR. Total system values were only calculated when both water and sediment phases were analysed.

At Level P-II, time-zero detections in the sediment were assigned to the water phase. At most time-points data of two replicate samples were available with use of individual values of replicates in the fitting procedures.

Software for kinetic evaluation

For total system kinetic evaluation (Level I), the software KinGUI v. 1.1 was used for fittings.

For kinetic evaluation at Level P-II, the software ModelMaker v. 3, with two individual compartments representing water and sediment, was utilised for fittings.

II. RESULTS AND DISCUSSION

The results of kinetic analysis at Level I showed that the SFO-SFO fits were visually and statistically acceptable. Therefore, the calculated half-lives for total-systems are considered as appropriate modelling endpoints following the respective FOCUS decision trees [*FOCUS (2006)*]. Total iprodione residues were found to degrade with a geometric mean half-life of 6.9 days and its major surface water metabolite RP 30228 was found to degrade with a geometric mean half-life of 63 days. A summary of the results for the two water-sediment systems is given in Table 7.2.2.3-8.

Table 7.2.2.3-8: Summary of the results of the kinetic calculations: total system degradation (Level P-I)

Water-sediment system	Kinetic model	Parent - iprodione		Metabolite - RP 30228	
		Half-life [days]	x^2-error [%]	Half-life [days]	x^2-error [%]
Iron Hatch	SFO-SFO	7.3	12.0	87.7	10.1
Mill Stream Pond	SFO-SFO	6.6	8.4	45.0	5.7
Geometric mean		6.9		63	

The results of the ModelMaker fits at Level P-II are summarised in Table 7.2.2.3-9. The χ^2-errors were acceptable for the water-phase but were significantly higher than the FOCUS guideline value of 15% in the sediment compartment. The t-test was failed due to a negative sediment-phase degradation rate (k_sed) in the Mill Stream Pond system. The results of the Level P-II analysis were therefore considered statistically unreliable.

Values for Fsed were calculated according to the FOCUS (2006) methodology, and were found to be consistent with the environmental fate data for iprodione in both aquatic systems. Since the Fsed check was passed at Level P-II but the SFO fit was not acceptable because the t-test was failed in at least one system, a conservative approach was used for selecting endpoints for model parameterisation according to FOCUS [*FOCUS (2006)*]. The total-system half-life calculated at Level P-I is used for the degrading compartment, and a conservative default half-life is used for the non-degrading compartment.

On the basis of this analysis, it is recommended to parameterise simulation models using the total-system iprodione half-life for the water-phase, and a conservative default half-life for the sediment-phase, when separate degradation rates are required for the water and sediment compartments.

Table 7.2.2.3-9: Summary of the results of the kinetic calculations for iprodione (Level P-II)

Water-sediment system	Water phase			Sediment phase			Fsed
	k_ads [d^{-1}]	k_wat [d^{-1}]	χ^2-error [%]	k_des [d^{-1}]	k_sed [d^{-1}]	χ^2-error [%]	
Iron Hatch	0.19089	0.07053	12.9	0.17553	0.11778	32.9	0.52
Mill Stream Pond	0.08631	0.16098	9.7	0.11952	-0.03117	36.7	0.42

III. CONCLUSION

The degradation of iprodione in aerobic water-sediment systems under laboratory conditions in the dark was evaluated according to the recommendations of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*].

The results of the kinetic analysis at Level I showed that SFO fits were visually and statistically acceptable, and therefore, the calculated half-lives for the total system are considered appropriate as modelling endpoints. For the sum of iprodione and its hydrolysis product RP 35606 the calculated geometric mean half-life was 6.9 days, and its major surface water metabolite RP 30228 was found to degrade with a geometric mean half-life of 63 days.

The results of kinetic analysis at level P-II to calculate separate half-lives for iprodione in water and sediment were found to be statistically unreliable. It is recommended to parameterise simulation models using the total-system half-life of iprodione for the water phase and a conservative default half-life for the sediment phase.

CA 7.2.2.4 Irradiated water/sediment study

No irradiated water/sediment study was performed. As iprodione does not adsorb light with a wavelength above 290nm and the compound is degraded rapidly by hydrolyses an effect of irradiation cannot be expected.

Overall summary on degradation rates in water/sediment systems

An overview of dissipation rates in water/sediment systems is given in Table 7.2.2.4-1.

The following considerations need to be taken into account for the endpoint selection:

The water-sediment study [*Purser (1992) – 1992/1001484*] was evaluated in Gurney (2008) [*Gurney (2008) –2008/1028646*] according to FOCUS kinetics [*FOCUS (2006)*]. Because of the reversibility of the reaction between iprodione and RP 35606, DT_{50} values could not be calculated for these two substances separately. Therefore, DT_{50} values were calculated for the degradation of the combined substances (iprodione + RP 35606) and used for PEC_{sw} and PEC_{sed} calculations for both iprodione and RP 35606. This approach is conservative since it overestimates the concentrations of the separate substances at any time point. For RP 35606, a maximum occurrence in water/sediment systems of 100% was considered as a worst-case assumption. Moreover, the DT_{50} values derived from the water/sediment study are more conservative than the DT_{50} derived from the hydrolysis study.

For iprodione and RP 35606, the level P-I modelling endpoint was selected during kinetic evaluation [*Gurney (2008) –2008/1028646*]. For modelling, the whole system $DegT_{50}$ is ascribed to either the water or the sediment phase, and a default value of 1000 days is ascribed to the other compartment. For compounds with K_{oc} between 100 and 2000 mL g^{-1}, as is the case for iprodione, running simulations and selecting the results that give the highest concentrations with both combinations is advised for ascribing the whole system $DegT_{50}$ and default value of 1000 days to the individual compartments. This was performed using one application scenario (fourfold application to root vegetables, [*Budde & Kallweit (2014) – 2013/1311392*]). Modelling endpoints were chosen accordingly (ascribing the whole system $DegT_{50}$ to the water phase, which resulted in higher PEC values). Further indication for choosing the water compartment as the degrading compartment can be derived from the hydrolysis study, where rapid iprodione degradation to RP 30228 can take place. An evaluation of hydrolysis studies [*Leake & Buntain (2000) – C022972*] also showed that the intermediate hydrolysis product RP 35606 is rapidly hydrolysed in water; therefore, the same settings as for iprodione were considered for the calculations.

Suplementary information – Scientific literature

A potential metabolite of iprodione in aquatic systems is 3-isopropylhydantoin (RP 30181). In the following study of Zadra (2006) the degradation of iprodione by yeast cells was investigated and compared to degradation in yeast-free medium. The yeast was able to cleave RP 30228 into dichloroaniline and RP 30181. In an alternative pathway RP 25040 was formed. In yeast-free medium the transformation took a different path and RP 35606 and RP 30228 were formed. This corresponds to the normal pathway found in the water/sediment study. Further degradation proceeds via ring-open metabolites, which can no longer form RP 30181. This indicates that some yeast strains can cleave RP 30228 under favourable conditions, but this is not a process found in the environment.

Report: CA 7.2.2.4/1
Zadra C. et al., 2006a
Biodegradation of the fungicide Iprodione by Zygosaccharomyces rouxii strain DBVPG 6399
2006/1050873
Guidelines: none
GLP: no

Executive Summary

The study was conducted in order to show the ability of an iprodione-resistant strain of the yeast *Zygosaccharomyces rouxii* to degrade iprodione at a concentration of 1 mg L^{-1}. Thus, the presence of iprodione and/or its biotransformation compounds were investigated in the distinct fraction of the culture medium (CM), washing liquid (WL), and raw extract (RE), and the degradation kinetics of the fungicide caused by the yeast cells was monitored. A control experiment in uncultured medium was also performed. The results showed a new degradation pathway of iprodione by the yeast leading to the formation of N-(3,5-dichlorophenyl)-2,4-dioxoimidazoline, 3-isopropylhydantoin, and 3,5-dichloroaniline, while in uncultured medium only the iprodione isomer was formed.

MATERIALS AND METHODS

1. Chemicals and iprodione degradation products

3,5-dichloroaniline (3,5-DCA), 3-isopropylhydantoin, and analytical grade iprodione (purity > 96%) were used, among other chemicals, for the study. The following degradation products were obtained:

- [N-(3,5-dichlorophenyl)-3-isopropyl-2,4-dioxo-1-imidazolidinecarboxamide] – Isomer of iprodione (obtained according to the procedure reported by Cooke et al. [*Cooke et al. (1979): The structural rearrangement of iprodione in ethanolic solution. Pestic. Sci.* ***10****, 393-398*]). Confirmed by GC-MS and NMR analyses.
- 3-(Isopropylcarbomyl)-5-(3,5-dichlorophenyl)hydantoic acid (obtained by chemical synthesis according to a method published by of Belafdal et al.[*Belafdal et al. (1989): Kinetic mechanism of cyclization in acidic media of* N-*[(3,5-dichloroanilino)carbonyl]-*N-*[(isopropylamino)carbonyl]glycine to hydantoins: iprodione and its isomer. J. Org. Chem.* ***54****, 4193-3198*]). Confirmed by NMR analyses.
- N-(3,5-dichlorophenyl)-2,4-dioxoimidazoline. Confirmed by GC-MS and NMR analyses.

2. Yeast strain and growth conditions

Twenty yeast strains from 17 species were tested for their ability to grow on YEPD plates (Yeast Extract Peptone Dextrose – medium for yeast growth) spiked with 1 and 10 mg/L iprodione. The yeast strain DBVPG 6399 was grown and maintained on YEPD (yeast extract 1 % w/v, peptone 1 % w/v, dextrose 2 % w/v) solidified with 1.7 % agar. Viability tests of cells growing on iprodione were carried out by methylene-blue staining followed by microscopical count.

3. Biodegradation studies of iprodione

For the biodegradation study, a YEPD-iprodione medium was prepared containing iprodione (10 % v/v acetone solution with 0.01 % Tween). Inoculum cells were obtained from a 24 h YEPD pre-culture, and were added to the YEPD-iprodione medium at a concentration of 10^7 cells/mL. Samples were taken at 0, 3, 6 and 9 days after inoculation and were centrifuged. After centrifugation of the medium (5 min at 3000 g), the supernatant, also called culture medium (CM), was collected and further processed. After additional washing and centrifugation of the cells of the CM, the supernatant washing liquid (WL) was collected. The cells contained in the pellet were broken open by repeated cell beating with glass beads at 1500 rpm for 2 min each time. This method resulted in > 95% of broken opened cells. After centrifugation (10 min at 3000 g), the supernatant, called raw extract (RE), was collected.

4. Iprodione chemical transformation study

The chemical transformation of iprodione was monitored in a YEPD-iprodione medium without cultured cells, referred to as uncultured medium (UM). The UM was incubated, sampled and processed according to the same procedures as for the CM.

5. Isolation of iprodione from samples

All samples (CM, WL, RE, and UM) were filtered (PTFE filter, 0.22 µm pore size), mixed with acetonitrile and then the two separate phases were analysed by HPLC or GC-MS. Recovery tests for CM samples were carried out for four fortification levels (range: 1 – 0.08 mg/L of iprodione). The mean percentage recovery was found to be 96.8 ± 0.5 % (n = 4).

6. HPLC, GC-MS, NMR and statistical analysis

HPLC analyses were performed on a Perking-Elmer PE 200 system equipped with an Inertsil 5 ODS-3 at a flow rate of 1 mL/min. The LOD were 0.05 mg L^{-1} for iprodione and 0.02 mg/L for 3,5-DCA and 3-isopropyldantoin. GC-MS analyses were performed on a Saturn II GC-MS system (Varian, Walnut Creek, CA). NMR spectra were obtained with a Bruker DRX-Advance 400 MHz spectrometer. Data sets were submitted to one-way analysis of variance. Standard errors of a mean were calculated and reported in graphs.

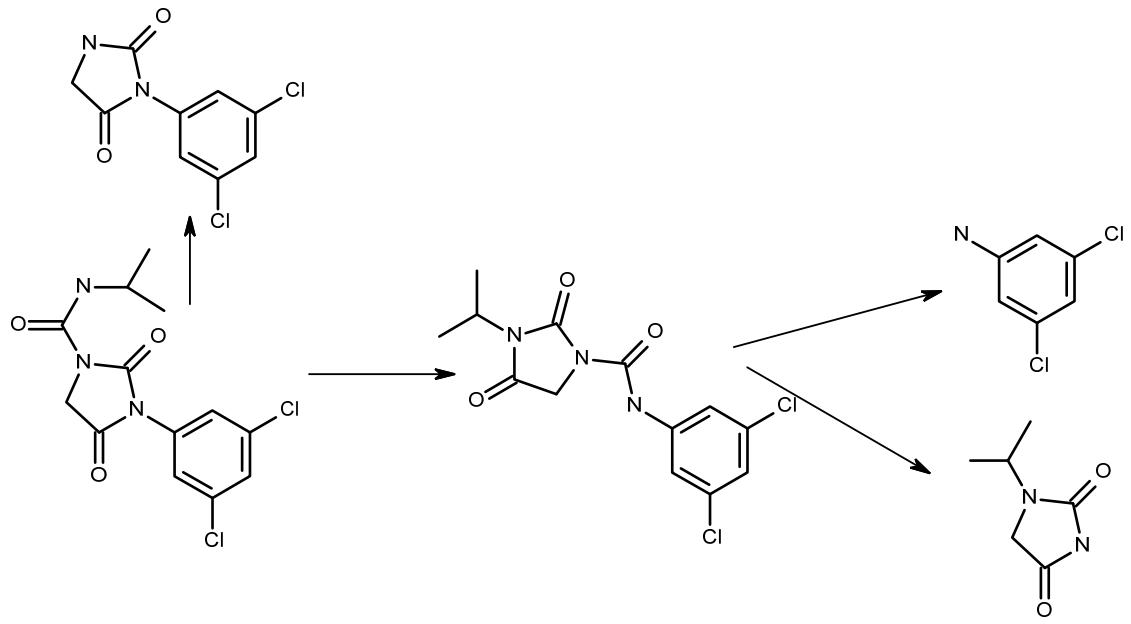
RESULTS AND DISCUSSION

Biotransformation of iprodione in CM

All strains, with one exception, were resistant to iprodione, suggesting that this fungicide is more active with fungi other than yeast. *Z. rouxii* DBVPG 6399 was chosen as representative of the resistant yeast strains; due to its high resistance to osmotic pressure, it could adapt to several extreme conditions in possible environmental biodegradations.

The presence of iprodione in the three culture fractions (CM, WL and RE) was monitored. Results showed that the concentration of iprodione decreased from 2.8 to 0.21 µmol/L in CM, gradually increased in RE from 0.20 to 0.38 µmol L^{-1}, and stayed almost constant in WL (at a very low concentration). The decrease of iprodione in CM could be due to a breakdown of the molecule by the yeast.

Over time, the yeast metabolised iprodione into different degradation products. The metabolites of iprodione were identified in the CM by using HPLC, GC-MS and NMR techniques. After 3 days of incubation, iprodione was metabolised to a structural isomer of iprodione and N-(3,5-dichlorophenyl)-2,4-dioxoimidazoline. Iprodione's isomer was then further transformed into 3,5-DCA and 3-isopropylhydantoin by cleavage of the amide bond of the isomer.



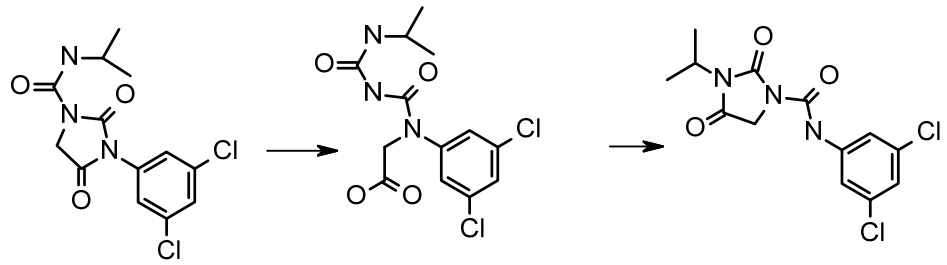
Transformation in cultured media

Kinetic evaluation of the degradation of iprodione by *Z. rouxii* showed that the fungicide disappeared at similar rates in CM and in biotic system CM + RE and that the half-lives were very similar (6 and 7 days, respectively). At the end of the experiment (after 9 days), up to 92 % of iprodione was metabolised. These results suggest that the yeast cells are not only able to degrade iprodione but can also take it up from the medium.

Transformation of iprodione in UM

To confirm the degradation pathway of iprodione by *Z. rouxii* experiments were carried out in YEPD solution with yeast cells and iprodione, YEPD solution with iprodione but without yeast cells and a buffered solution with iprodione.

The half-life of iprodione in the UM was ca. 5 days; the concentration decreased rapidly from 0.43 to 0.05 µmol/L (after 9 days, 83% of the fungicide had disappeared). In the UM and in the buffer solution iprodione was transformed into a new intermediate compound: 3-(isopropylcarbomyl)-5-(3,5-dichlorophenyl)hydantoic acid, which was then converted into a stable structural isomer of iprodione [N-(3,5-dichlorophenyl)-3-isopropyl-2,4-dioxo-1-imidazolidinecarboxamide].



Transformation in uncultured media

The kinetic data from the experiment with UM coincide with data reported in literature. Also, the transformation process with the isomer of iprodione as final transformation product has also been found in other biotic and abiotic systems such as water and soil (European Commission, 2002). It is important to point out that during the degradation in soil and water environments iprodione does not transform into breakdown products but undergoes only a structural rearrangement.

CONCLUSION

The results of the study show that the yeast *Z. rouxii* DBVPG 6399 is resistant to iprodione and is even able to degrade the fungicide. The extensive degradation of iprodione by the yeast leaded to the formation of three transformation products: N-(3,5-dichlorophenyl)-2,4-dioxoimidazoline (RP 25040), 3-isopropylhydantoin (RP 30181) and 3,5-DCA. In contrast under yeast-free conditions only the isomer of iprodione was formed and no cleavage to §-isopropylhydantoine and 3.5 DCA was observed.

Iprodione is very unstable under alkaline conditions. Morales (2013) investigated the influence of humic substances on the degradation rate.

Report:	CA 7.2.2.4/2 Morales J. et al., 2013b Stability study of Iprodione in alkaline media in the presence of humic acids 2013/1414998
Guidelines:	none
GLP:	no

Executive Summary

The aim of the study was to investigate the influence of humic aggregates in water solution upon the chemical stability of iprodione under basic conditions. Humic substances (HSs) represent a large portion of organic matter in natural environments and soil. The role of humic acids (one of these humic substances) and their influence on the hydrolysis of iprodione (one of the major transformation pathways) has been evaluated during this study.

In order to determine how humic acids can modify the efficiency of hydrolysis of Iprodione, experimentally observed rate constants were analysed in terms of kinetic models. Kinetic coefficients of the catalytic process were obtained in order to model the kinetic behaviour of iprodione.

Results show an inhibition of the alkaline hydrolysis of iprodione which was rationalised in terms of the micellar pseudophase model. Subsequently, these results were compared with the corresponding results in the same natural colloidal aggregates in the presence of other pesticides.

MATERIALS AND METHODS

1. Reagents

3-(3,5-dichlorophenyl)-N-isopropyl-2,4-dioxoimidazolidine-1-carboxamide (iprodione) and Panreac reagents (sodium hydroxide and acetonitrile) were used in the study. Humic aggregates (HAs) were isolated from soil according to the method described in "Methods of Soil Analysis" [*Methods of Soil Analysis (1999). American society of Agronomy. Madison, WI, USA*].

2. Kinetic measurements and data analysis

All kinetic tests were conducted under pseudo first-order conditions ([IP] « [OH^-]). Reactions were monitored through the first-order basic hydrolysis of iprodione using a Varian Cary 50 Bio spectrophotometer with the observation cell thermostated at 25.0 ± 1°C. To monitor the alkaline hydrolysis, the reactions were measured at 248 nm and because HAs absorb in the UV-vis region, the spectrum of HAs in absence of a reaction was used as blank. Nonlinear regression was carried out using the commercial package Profit 6.2 (QuantumSoft).

The rate of disappearance of iprodione was calculated according to the following equation:

Equation 1 Rate of disappearance of iprodione

$$v = -\frac{d[IP]}{dt} = k_w[IP]_t[OH^-]_t = k_{obs}[IP]_t = k_{obs}([IP]_0 - [products])$$

with [IP] being the concentration of iprodione, k_w being the biomolecular rate and k_{obs} being the pseudo-first rate constant for the basic hydrolysis of iprodione.

The concentrations in terms of absorbance have been calculated by integrating Equation 1:

Equation 2 Concentrations in terms of absorbance

$$A_t = A_0 exp(-k_{obs}t) + A_\infty\left(1 - exp(-k_{obs}t)\right)$$

with A_t, A_0 and A_∞ being the absorbance at times zero, t and infinity, respectively.

RESULTS AND DISCUSSION

The basic hydrolysis of iprodione has been analysed in the presence of HAs. A 2-fold inhibition was observed in the degradation of iprodione in presence of HSs micellar aggregates, representing an increase of the half-life of iprodione. This inhibition is due to the association of the humic substances to iprodione.

Humic acid polymers aggregate spontaneously in water, forming micelle-like structures. A single polymer chain arranges itself in a manner that exposes its hydrophilic parts (*e.g.*, carboxy groups) to the aqueous surroundings, while isolating its hydrophobic portion in the centre of the structure.

The clear inhibition of the hydrolysis of iprodione due to increasing humic acid concentrations is probably caused by the fact that the hydrolysis rate in the dispersed phase is significantly slower than in the continuous phase (water).

Since the surface charge of humic acid is negative, taking into account simple electrostatic considerations, it can be assumed that the dispersed phase plays a role by compartmentalisation of the reagents preventing the contact between iprodione and OH^- in the dispersed phase and thus, the hydrolysis of iprodione is inhibited by the OH^- exclusion from the surface of the aggregate.

Taking into account these considerations, the basic hydrolysis reaction of iprodione can be modelled by the two-pseudophase model formed by HAs micellar aggregates and water. The kinetic results observed during the study imply a large increase of iprodione's half-life due to the effect of the hydrolysis inhibition inside the HSs aggregates. The pseudophase model was proved to predict the kinetic behaviour of iprodione in humic aggregates satisfactorily and thus, represents a useful tool for a quantitative analysis of these kinetic measurements.

The inclusion constant of iprodione between micellar and water phases calculated in humic acids was higher than the one from other reactions studied with other pesticides, which could probably be explained by the hydrogen bonding of iprodione with the phenolate and carboxylate groups in the interface of the HSs micellar aggregates. Finally, it could be affirmed that hydrophobicity of the humic acid core was the main force of interaction between these natural colloidal aggregates and iprodione.

CONCLUSION

The inhibitory effects of humic substances on the basic hydrolysis of iprodione was investigated. It has been observed that the presence of micelle-like colloids as HAs in restricted media implied an inhibition of the basic hydrolysis of iprodione (2-fold) which lead to an increase of the half-life of iprodione of approximately 100 %.

Comparing the behaviour of iprodione and other substances in presence of the same natural colloidal aggregates it has been found that the binding between iprodione and the humic substance is higher than the binding observed between the humic substance and other pesticides. These differences in inhibition behaviour could be attributed to the differences in hydrophobicity and hence in their penetrability inside HAs aggregated core.

CA 7.2.3 Degradation in the saturated zone

From leaching simulation modelling it is not expected that iprodione or its metabolites will leach into the saturated zone. Therefore no additional study is required.

Overall summary on degradation rates in water/sediment systems

An overview of dissipation rates in water/sediment systems is given in Table 7.2.2.4-1.

The following considerations need to be taken into account for the endpoint selection:

The water-sediment study [*Purser (1992) – 1992/1001484*] was evaluated in Gurney (2008) [*Gurney (2008) –2008/1028646*] according to FOCUS kinetics [*FOCUS (2006)*]. Because of the reversibility of the reaction between iprodione and RP 35606, DT_{50} values could not be calculated for these two substances separately. Therefore, DT_{50} values were calculated for the degradation of the combined substances (iprodione + RP 35606) and used for PEC_{sw} and PEC_{sed} calculations for both iprodione and RP 35606. This approach is conservative since it overestimates the concentrations of the separate substances at any time point. For RP 35606, a maximum occurrence in water/sediment systems of 100% was considered as a worst-case assumption. Moreover, the DT_{50} values derived from the water/sediment study are more conservative than the DT_{50} derived from the hydrolysis study.

For iprodione and RP 35606, the level P-I modelling endpoint was selected during kinetic evaluation [*Gurney (2008) –2008/1028646*]. For modelling, the whole system $DegT_{50}$ is ascribed to either the water or the sediment phase, and a default value of 1000 days is ascribed to the other compartment. For compounds with K_{oc} between 100 and 2000 mL g^{-1}, as is the case for iprodione, running simulations and selecting the results that give the highest concentrations with both combinations is advised for ascribing the whole system $DegT_{50}$ and default value of 1000 days to the individual compartments. This was performed using one application scenario (fourfold application to root vegetables, [*Budde & Kallweit (2014) – 2013/1311392*]). Modelling endpoints were chosen accordingly (ascribing the whole system $DegT_{50}$ to the water phase, which resulted in higher PEC values). Further indication for choosing the water compartment as the degrading compartment can be derived from the hydrolysis study, where rapid iprodione degradation to RP 30228 can take place. An evaluation of hydrolysis studies [*Leake & Buntain (2000) – C022972*] also showed that the intermediate hydrolysis product RP 35606 is rapidly hydrolysed in water; therefore, the same settings as for iprodione were considered for the calculations.

For iprodione and RP 35606, the geometric mean total system half-life of 6.9 days was selected to represent degradation in both the water and the sediment phase at Step 1. At Step 2 to 4, the DT_{50} value for the water phase was set to the geometric mean total system half-life of 6.9 days, while the DT_{50} for the sediment phase was set to a conservative default of 1000 days according to FOCUS [*FOCUS (2012): Generic guidance for FOCUS surface water Scenarios, version 1.2. (December 2012), 357 pp*].

For RP 30228, the geometric mean total system half-life of 63 days was assigned to the sediment phase (both water and sediment at Step 1) and a conservative default DT_{50} of 1000 days was assigned to the water phase (at Step 2-3), in line with the FOCUS recommendation [*FOCUS (2001): FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC. - Report of the FOCUS Working Group on Surface Water Scenarios. EC Document Reference SANCO/4802/2001-rev.2 final (May 2003), 238 pp, page 204*].

Table 7.2.2.4-1: Summary of degradation rates in water/sediment systems: total system degradation (Level P-I)

Water-sediment system	Kinetic model	Iprodione with RP 35606	Metabolite - RP 30228
		DT_{50} [d]	DT_{50} [d]
Iron Hatch	SFO-SFO	7.3	87.7
Mill Stream Pond	SFO-SFO	6.6	45.0
Geometric mean		6.9	63

CA 7.3 Fate and behaviour in air

Based on its physical-chemical properties, iprodione has no potential for volatilisation (vapour pressure 5 x 10^{-7} Pa at 25°C). Two old volatilisation studies exist [*Kubiak (1992) – C021255; Jendrzejczak (1992) – C022518*], but have experimental deficiencies.
Three other studies were done to replace the former and were presented in the original Annex II dossier. They are shortly summarised herebelow. No new studies were performed to address the behaviour of iprodione in air.

CA 7.3.1 Route and rate of degradation in air

Study presented in the original Annex II Dossier (1995):

Report: Maestracci, M. (1994): Iprodione. Estimation of the rate of photochemical transformation in the atmosphere under tropospheric conditions (Study 94-34).
Report Rhône-Poulenc R&D/CRLD/AN/9416338 of September 19, 1994.
C022518
GLP: Yes

The rate of photochemical transformation of iprodione in the atmosphere under tropospheric conditions was estimated according to the method described by the OECD [*OECD Environment Monograph no 61 (1992): The rate of photochemical transformation of gaseous organic compounds in air under tropospheric conditions*].

Results show that iprodione is removed rapidly by photoreaction with OH-radicals. The value of the reaction rate constant at 298K is 1356.315 x $10^{-7}s^{-1}$ which corresponds to a half-life of about 85 daylight minutes. The main reaction will be probably an interaction of OH-Radicals with > NH and >N-groups.

CA 7.3.2 Transport via air

Studies presented in the original Annex II Dossier (1995):

Report: Jendrzejczak, N. et al. (1993): Soil surface volatility study of iprodione formulated as Verisan (suspension concentrate (SC) containing 255 g/l iprodione).
Report Rhône-Poulenc R&D/CRLD/AN no 9315034 of January 21, 1993.
C023337
GLP: Yes

Method: A soil surface volatility study with radiolabelled iprodione formulated as 'Verisan' (German trade name of the 255 g/L suspension concentrate which code number is EXP.1861) was carried out according to the BBA Guidelines IV, 6-1, at 20.5°C. A dose of 177.06 kBq corresponding to an application rate of 645 g of iprodione per hectare was applied to the surface of a German standard soil (LUFA 2.1) in a volatilisation chamber fitted with an air sampling device. Samples were taken 1, 3 6 and 24 hours after application.

Results: No radioactivity could be detected in air (LOD = 0.07% of applied). After 24 hours 99.02% of the applied radioactivity was recovered from soil. Less than 0.1% was found in the air, about 0.1% was found on the volatilisation chamber and 98.8% was found in the soil. It can be assumed that less than 0.1 % of iprodione applied is volatilised from soil surface within 24 hours. This showed the lack of volatility of iprodione under these conditions.

Report: Jäger, J. & Kubiak, R. (1993): Volatilization of ^{14}C-iprodione formulated according to Verisan from plant surfaces under laboratory conditions (BBA Richtlinien Teil IV6-1).
Report LLFA RPA 05 (R&D/CRLD/AN/9315104 of January 13, 1993.
C023335
GLP: Yes

Method: A study on the volatility of radiolabelled iprodione formulated as 'Verisan' from plant surfaces was carried out according to BBA Guidelines IV, 6-1, at 20.1°C. The simulated application rate was 765 g/ha. The experiment was carried out in duplicate. After deduction of the application losses and the amount applied to the soil cover 725.1 or 953.7 kBq were applied to the plants in a volatilisation chamber fitted with an air sampling device. Samples were taken after 1, 3, 6 and 24 hours.

Results: The radioactivity measured in the air absorption system was below LOD (100 dpm = 0.0017 kBq) at all sampling times. After 24 hours the amount of radioactivity in the air was less than the limit of detection and all recovered radioactivity was associated with the plants. 119.7 % of calculated applied activity were recovered from the plants in trial 1 and 102.7% in trial 2.

Conclusion: No volatilisation of ^{14}C iprodione occurs from plant surfaces in the course of 24 hours.

CA 7.3.3 Local and global effects

The degradation rates for reactions of BAS 610 F - iprodione with OH radicals and ozone in the atmosphere were calculated using the method based on ATKINSON's increment method (see summary CA 7.3.1, DocID C022518). Based on the resulting degradation rate of k_{OH} = 271.263.28 10^{-12} cm^3 $molecule^{-1}$ s^{-1}, the atmospheric degradation half-life of the substance via this reaction route is $t_{1/2}$ = 85 min. Although O_3 is also likely to react with iprodione, the degradation rate resulting from ozone attack could not be estimated by the OECD method due to the lack of increments.

Hence, iprodione is not listed in Annex I of Regulation (EC) 2037/2000 on substances that deplete the ozone layer and iprodione does not belong to the greenhouse gases listed in P Forster, PV Ramaswamy et al. (2007): Changes in Atmospheric Constituents and in Radiative Forcing; in: Climate Change 2007: The Physical Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on climate Change.

CA 7.4 Definition of the residue

Within the last registration process iprodione and PR 30228 were considered to be the only relevant residue for soil, groundwater and surface water and iprodione for air. However, based on the results of the new studies, it is necessary to adjust the definition of the residue for the individual environmental compartments.

CA 7.4.1 Definition of the residue for risk assessment

According to the results presented in chapters 7.1 – 7.3 the following compounds have to be considered for the environmental risk assessment:

Soil: Iprodione, soil metabolites RP 35606, RP 30228, RP 25040, RP 36221, RP 44247 and dichloroaniline (RP 32596)

Groundwater: Iprodione, soil metabolites RP 35606, RP 30228, RP 36221, dichloroaniline (RP 32596) and soil photolysis metabolites RP 25040 and RP 44247 (LS720942).
Based on the medium adsorption to soil the parent molecule iprodione has a moderate leaching potential. This is more pronounced in acidic soils as the degradation time increases at pH levels below 6.
The predicted annual leachate concentrations of the soil metabolites were < 0.1 µg L^{-1} in all scenarios calculated with FOCUS-PEARL 4.4.4. Calculations with FOCUS-PELMO 5.5.3 were also below 0.1 µg L^{-1}, with the exception of metabolite RP 35606, for which the trigger value of 0.1 µg L^{-1} was exceeded in two out of 46 scenarios in the Tier 1 calculations for acidic soils, with a maximum of 0.136 µg L^{-1} in the scenario Porto for carrots (late application). Tier 2 calculations were performed for acidic soils, resulting in values below 0.1 µg L^{-1} in all tested scenarios *[see MCP chapter 9.2.4]*.

The leaching of unacceptable amounts of iprodione and its metabolites RP 25040, RP 35606, RP 30228, RP 36221, dichloroaniline (RP 32596) and RP 44247 (LS 720942) after application to carrots and lettuce (surrogate crop: cabbage) is highly unlikely.

Surface water: Iprodione, metabolites RP 35606, RP 30228, RP 36221, dichloroaniline (RP 32596) and soil photolysis metabolite RP 25040.

Sediment: Iprodione, RP 35606 and RP 30228

Air: Iprodione

CA 7.4.2 Definition of the residue for monitoring

Soil: Iprodione, soil metabolites RP 30228, RP 36221 and dichloroaniline (RP 32596)

Groundwater: Iprodione, soil metabolites RP 35606, RP 30228

Surface water: Iprodione, metabolites RP 35606, RP 30228

Sediment: Iprodione, RP 35606 and RP 30228

Air: Iprodione

CA 7.5 Monitoring data

Introduction

Publically available ground and surface water monitoring data were checked for analyses and findings of iprodione and the metabolites RP 30228, RP 36221 and RP 32596. No data were found for the metabolites in either ground or surface water. No surface water monitoring data was found for Iprodione. Data for iprodione in ground water were found for France, Czech Republic, Denmark, Italy and the Netherlands. These data are summarized in Table 7.5-1 to Table 7.5-5.

Results and discussion

No exceedances of the regulatory trigger concentration of 0.1 µg/L occur in the data from Czech Republic (4 years with 39 monitored wells each year) Denmark (3 years with limited number of wells) and Netherlands (3 years, between 52 and 184 wells monitored per year). In Italy a single exceedance of the trigger is seen from four monitoring years with between 361 and 865 wells monitored per year.

The most comprehensive dataset was that for France (ADES national groundwater database) with monitoring data available for groundwater from 1992 onwards. The number of sampled wells increased from year to year. Since 2009 samples from more than 5000 wells per year have been analysed for Iprodione. To provide a current picture, latest findings are discussed in the following. The most recent exceedances of the groundwater trigger concentration were in 2011, where concentrations > 0.1 µg/L were detected at 4 monitoring wells and in 2012 at 1 well (which also had an exceedance in 2011). The measured concentrations for three of these wells are shown in Figure 7.5-1. Measured concentrations below the limit of quantification (LOQ) are assigned a value of 0.5 x LOQ in the figure. It can be seen that the detections of Iprodione occur in discrete peaks (potentially from point sources) and do not indicate that there is a plateau concentration in groundwater resulting from regular applications of the substance. At the fourth well only two measured concentrations are available in the database, the first in 2009 being < LOQ, the second in 2011 with 11.26 µg/L, which – if correct – would strongly indicate a point source contamination.

Table 7.5-1: Monitoring data for iprodione in ground water available for France

Country	**FR**				
Data Source	**ADES Database (http://www.ades.eaufrance.fr)**				
Data for years	**1992 - 2013**				
Year	**Number of wells sampled**	**Number of analyses**	**Number of detections > LOQ**	**Number of detections >0.1 µg/L**	**Number of wells with detections >0.1 µg/L**
1992	57	77	0	0	0
1993	64	152	0	0	0
1994	47	111	0	0	0
1995	38	95	0	0	0
1996	34	34	0	0	0
1997	162	433	0	0	0
1998	162	236	0	0	0
1999	*65*	*111*	*0*	*0*	*0*
2000	*101*	*153*	*2*	*2*	*2*
2001	374	701	0	0	0
2002	465	1331	2	1	1
2003	943	1831	2	1	1
2004	1276	2216	3	3	3
2005	2274	4118	7	2	2
2006	2543	4811	12	3	2
2007	2918	5482	7	2	2
2008	2557	5756	6	0	0
2009	5253	9515	11	2	2
2010	5344	10498	4	0	0
2011	5315	10800	9	4	4
2012	5978	11394	18	1	1
2013	2755	4446	2	0	0

Table 7.5-2: Monitoring data for iprodione in ground water available for Czech Republic

Country	**CZ**				
Data Source	**CHMI database (http://hydro.chmi.cz/isarrow/)**				
Data for years	**2009 - 2012**				
Year	**Number of wells sampled**	**Number of analyses**	**Number of detections >LOQ**	**Number of detections >0.1 µg/L**	**Number of wells with detections >0.1 µg/L**
2009	39	39	0	0	0
2010	39	39	0	0	0
2011	39	39	0	0	0
2012	39	39	0	0	0

Table 7.5-3: Monitoring data for iprodione in ground water available for Denmark

Country	**DK**				
Data Source	**Jupiter Database (http://jupiter.geus.dk)**				
Data for years	**1995, 1996, 2011**				
Year	**Number of wells sampled**	**Number of analyses**	**Number of detections >LOQ**	**Number of detections >0.1 µg/L**	**Number of wells with detections >0.1 µg/L**
1995	21	21	0	0	0
1996	1	1	0	0	0
2011	7	7	0	0	0

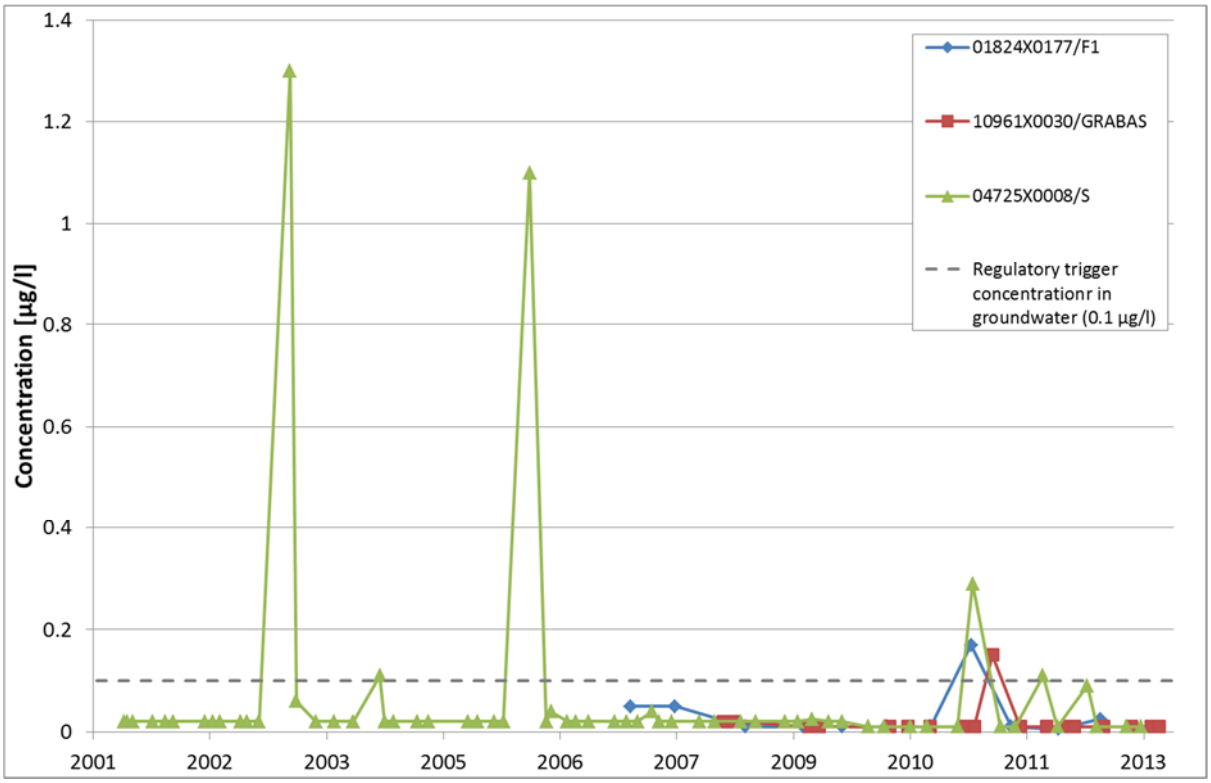
Table 7.5-4: Monitoring data for iprodione in ground water available for Italy

Country	**IT**				
Data Source	**ISPRA**				
Data for years	**2007 - 2010**				
Year	**Number of wells sampled**	**Number of analyses**	**Number of detections >LOQ**	**Number of detections >0.1 µg/L**	**Number of wells with detections > 0.1 µg/L**
2007	661	1286	1	0	0
2008	880	1533	0	0	0
2009	865	1402	3	3	1
2010	361	652	3	0	0

Table 7.5-5: Monitoring data for iprodione in ground water available for Netherlands

Country	**NL**				
Data Source	**Monitoring programs of provincial authorities and water authorities**				
Data for years	**2003, 2007, 2012**				
Year	**Number of wells sampled**	**Number of analyses**	**Number of detections >LOQ**	**Number of detections >0.1 µg/L**	**Number of wells with detections >0.1 µg/L**
2003	52	53	0	0	0
2007	178	188	0	0	0
2012	184	214	1	0	0

Figure 7.5-1: Measured concentrations of Iprodione in groundwater at monitoring wells in France where concentrations > 0.1 µg/L have been detected in recent years (source: ADES database http://www.ades.eaufrance.fr)



Conclusion

Publically available groundwater monitoring data for iprodione shows that, following application according to the label, the leaching of unacceptable amounts of iprodione is highly unlikely.

Supplementary Information – Scientific literature
While monitoring data for groundwater were taken from databases reflecting a systematic monitoring for many years the following studies of Schummer rely on samples from a single site and cover a limited time. In two studies Schummer investigates the occurance of pesticides in air during application season. While the first report is focused on the analytical method and the findings, which were compared to an earlier study from 2002, the second report investigates the partitioning of the pesticides between gas and particle phase using the same analytical results.

Report:	CA 7.5/1 Schummer C. et al., 2009a Temporal variations of concentrations of currently used pesticides in the atmosphere of Strasbourg, France 2009/1129362
Guidelines:	none
GLP:	no

Executive Summary

The behaviour of 71 pesticides including iprodione was monitored in Strasbourg, an intensive farming region in the northeast of France. Atmospheric samples were collected during the period from April 18 to May 29, 2007.

Gas and particle phases were sampled together by a high-volume sampler on the basis of a 48 h sampling interval. Both phases were extracted separately. Pesticide concentrations were measured with gas chromatography equipped either with an MS/MS or an ECD detector.

Iprodione was detected in the analysed air samples at up to 42.31 ng m^{-3} in all samples. The effect of time, temperature, atmospheric pressure and humidity on temporal variations of pesticide concentrations in air was investigated. A correlation with time (study duration), temperature, atmospheric pressure or humidity was found for some pesticides, but correlation with time was not significant for the concentrations of iprodione in air.

I. MATERIALS AND METHODS

1. Test material

Pesticide standards including iprodione were used.

2. Test sites

Samples were taken in Strasbourg, a region of intensive farming in the northeast of France. Corn and grapes are the most important crops in this region. They formed about 60% of the total agricultural land use in 2000.

3. Sampling and analysis

Air samples were taken by means of an in-laboratory developed high-volume sampler during the period from April 18 to May 29, 2007. Samples were collected on a basis of a 48 h interval. The sampler was placed on a distance of 5 km from field crops, about 0.5 km from the city centre, and 2 km from industrial zones.

Gas samples and particulates were collected at the same time on a glass fibre filters (Whatman, GF/A), ø = 30 cm) and 20 g resin XAD-2 (Supelco) at a flow rate of 9.96 L min^{-1}. Before sampling, the filter and the resin were soaked for 24 h with 50:50 n-hexane/CH_2Cl_2 and dried. After that they were wrapped individually in aluminium foil or clean plastic bags, and stored in the dark at -20°C until used.

Pesticides were extracted from the filters and resin separately with 50:50 n-hexane/CH_2Cl_2 for 20 h (Soxhlet extraction). After that, extracts were concentrated to about 1 mL using a rotary evaporator. Samples were spiked with internal standard.

Samples were analysed for pesticides using gas chromatography coupled with either mass spectrometry with ion trap (MS/MS) or electron capture detector (ECD) depending on the physic-chemical properties of the pesticide. Iprodione was quantified by GC-2ECD, with a limit of detection (LOD) of 0.1 pg m^{-3}.

The results of both phases (gas and particle) were combined to obtain the pesticide concentrations found in the total atmosphere.

4. Quality control

Interday and intraday accuracies and variabilities in the concentrations of all studied pesticides, measured by GC-MS/MS or GC-ECD were calculated. Interday and intraday accuracies for GC-MS/MS analysis varied between 92.1% to 118.3%, and 97.3% to 109.7%, and variations ranged from 3.2% to 8.7% and from 7.9% to 16.8%, respectively. For GC-ECD analysis, interday and intraday accuracies were between 89.1 to 120.1% and 93.4% to 111.2%, whereas the variations in interday and intraday measurements ranged from 8.4% to 14.4% and 2.9% to 7.9%, respectively.

II. RESULTS AND DISCUSSION

Findings

The results of this study showed that iprodione was detected in all analysed samples collected in Strasbourg. The concentration of iprodione ranged from 3.84 to 42.31 ng m^{-3}, with an average of 11.12 ± 11.08 ng m^{-3}. The results also showed that about 70% of iprodione was detected in the extracts of the particle phase of the sampled air.

In addition, results were compared with the results from an identical study conducted on 27 pesticides with 18 samplings between March 24 and June 19, 2002. Iprodione was not detected at all in 2002, while concentrations in 2007 were important with a maximum concentration of 42.31 ng m^{-3} and an average concentration of 11.12 ng m^{-3}.

III. CONCLUSION

Iprodione was detected in all air samples collected in the intensive farming region of Strasbourg (France). When the results were compared to a study from 2002, it was evident that iprodione was not found in that year but was present at higher concentrations in 2007. A correlation with time (study duration), temperature, atmospheric pressure or humidity was found for some pesticides, but correlation with time was not significant for iprodione concentrations in air. The maximum concentration of iprodione detected in the analysed air samples was 42.31 ng m^{-3}.

Report: CA 7.5/2
Schummer C. et al., 2010a
Gas/particle partitioning of currently used pesticides in the atmosphere of Strasbourg (France)
2010/1229752
Guidelines: none
GLP: no

Executive Summary

In this study the behaviour of 71 pesticides including iprodione was monitored in Strasbourg, an intensive farming region in the northeast of France. Air and particle samples were collected during the period from April 18 to May 29, 2007 in order to study the gas/particle-partitioning of the pesticides and to evaluate the influence of environmental parameters (temperature and humidity) on this partitioning.

Samples were taken by a high-volume sampler on the basis of a 48 h sampling interval. Both phases were extracted separately. Pesticide concentrations were measured with gas chromatography equipped either with MS/MS or ECD detector.

Iprodione was detected in all samples analysed for the particle phase (n = 10). The concentration of iprodione ranged from 3.68 to 38.92 ng/m^3, with an average of 8.36 ± 10.80 ng/m^3. For the gas phase, iprodione was detected in 8 samples out of 10. The concentration of iprodione in the gas phase ranged from 2.46 to 5.97 ng/m^3, with an average value of 3.45 ± 1.06 ng/m^3.

Gas/particle partitioning of iprodione was influenced by temperature ($p<0.05$), but not by relative humidity. Results also suggested that adsorption has an influence on partitioning.

I. MATERIALS AND METHODS

1. Test material

Pesticide standards including iprodione.

2. Test sites

Samples were taken in Strasbourg, an intensive farming region in the northeast of France.

3. Sampling and analysis

Air and particle samples were taken during the period between April 18 and May 29, 2007 by means of a high-volume sampler equipped with a filter-resin plug. Particle and gas phase samples were collected simultaneously on 30-cm-diameter glass fibre filters and 20 g XAD-2 resin, a copolymer of styrene/divinylbenzene and macro porous acrylic ester. Prior to sampling, filters and resins were cleaned in a Soxhlet apparatus for 24 h using 50:50 n-hexane/CH_2Cl_2.

Ten air samples were collected based on a 48 h sampling interval with a flow rate of 10 m^3 h^{-1}. The sampler was placed at a distance of 5 km from field crops, about 0.5 km from the city centre, and 2 km from industrial zones.

Pesticides were extracted from the filters and resins separately with 50:50 n-hexane/CH_2Cl_2 for 20 h by Soxhlet extraction. Afterwards, extracts were concentrated to about 1 mL using a rotary evaporator. Samples were spiked with an internal standard (tecnazen).

Samples were analysed for pesticides using gas chromatography coupled with either ion-trap mass spectrometry (GC-MS/MS) or electron capture detection (GC-2ECD) depending on the physical and chemical properties of the pesticides. For iprodione, analyses were performed with GC-2ECD. The detection limit was 0.1 pg/m^3.

4. Quality control

Three replicates of control samples were analysed at two different concentrations to determine intraday precision, while the interday accuracy was determined for control samples on three separate days.

II. RESULTS AND DISCUSSION

Findings

Iprodione was detected in all samples analysed for the particle phase (n = 10). The concentration of iprodione ranged from 3.68 to 38.92 ng/m^3, with an average of 8.36 ± 10.80 ng/m^3. For the gas phase, iprodione was detected in 8 samples out of 10. The concentration of iprodione in the gas phase ranged from 2.46 to 5.97 ng/m^3, with an average value of 3.45 ± 1.06 ng/m^3.

Gas/particle partitioning of iprodione was influenced by temperature ($p<0.05$), but not by relative humidity. Results also suggested that adsorption has an influence on partitioning.

III. CONCLUSION

Iprodione was detected with a higher frequency and concentration in the particulate phase than in the gas phase in the analysed samples. The authors observed that temperature variations have an effect on phase partitioning of the monitored pesticides. Particle bound pesticides can be removed from air by dry deposition or rain.

Report: CA 7.5/3
Mansilha C. et al., 2011a
Groundwater from infiltration galleries used for small public water supply systems: Contamination with pesticides and endocrine disruptors
2011/1296191
Guidelines: none
GLP: no

Executive Summary

In this study the behaviour of 31 compounds (mainly pesticides, e.g. iprodione) was monitored in water samples from infiltration galleries in small villages near the Douro River (North Portugal). Samples were collected in July 2009 and analysed by GC-MS.

The general idea that water from infiltration galleries always presents high quality was not confirmed. Iprodione, however, was not detected in any of the samples.

I. MATERIALS AND METHODS

1. Test material

Pesticide standards (purity >98%) including iprodione.

2. Test sites

Infiltration galleries are an old means to prepare drinking water from surface water. In an investigation of the water quality prepared by infiltration, nine water samples were taken from small public fountains of infiltration galleries in small villages in the Alto Douro Demarcated Wine region (North of Portugal) near the Douro River.

3. Sampling and analysis

Water samples were taken in July 2009. Prior to extraction, 500 mL volume of water samples were filtered through glass fibre filters and spiked with an internal standard. Samples were extracted by solid phase extraction using methanol and acetonitrile, evaporated and re-suspended in 500 µL methanol for analysis by GC-MS. The detection limit (LOD) for iprodione was 6.0 ng/L, the limit of quantification (LOQ) was 19.9 ng/L.

II. RESULTS AND DISCUSSION

Findings

A total of 12 out of 31 compounds (including pesticides and estrogens) were detected in the water samples analysed. Four of them (i.e. folpet, terbuthylazin-desethyl, dimethoate and terbuthylazin) were found above their LOQ and two of the analysed compounds (terbuthylazin-desethyl and terbuthylazn) were even detected at concentrations exceeding the 0.1 µg/L EU limit for individual pesticides. Iprodione was not detected in any of the samples.

III. CONCLUSION

The general idea that water from infiltration galleries always presents high quality was not confirmed. Iprodione, however, was not detected in any of the samples.

Report: CA 7.5/4
Scheyer A. et al., 2005a
Analysis of trace levels of pesticides in rainwater using SPME and GC-tandem mass spectrometry
2006/1050894
Guidelines: none
GLP: no

Executive Summary

In this study the behaviour of 20 pesticides including iprodione was monitored at two sampling sites in Alsace (eastern France): Strasbourg, an intensive farming region in the northeast of France, representing an urban area, and Erstein (25 km southeast of Strasbourg), representing a rural area. Samples at the two sites were collected simultaneously on a weekly basis between January 2002 and September 2003.

Samples were extracted using solid phase microextraction (SPME) and analysed by gas chromatography coupled with ion-trap tandem mass-spectrometry (GC-ITD-MS/MS). The parameters that can affect the SPME process were optimised during method evaluation.

Iprodione was rarely detected in rainwater samples from Strasbourg and Erstein and concentrations were always below the detection limit.

I. MATERIALS AND METHODS

1. Test material

Pure pesticide standards including iprodione (purity: 99.0%).

2. Test sites

Samples were taken in Strasbourg, an intensive farming region in the northeast of France, representing an urban area, and in a rural area, represented by the small town of Erstein (25 km southeast of Strasbourg).

3. Sampling and analysis

Samples were collected simultaneously at the two sites on a weekly basis between January 2002 and September 2003 using a wet-only rainwater sampler. The sampler for the urban area was placed in the botanical gardens of Strasbourg, with intensive agricultural activity (essentially maize farming) taking place 15 km south of the city centre. The sampler for the rural area was placed on the soil 2 km off the small town Erstein in 300 m distance to an area treated with pesticides.

Pesticides were extracted using solid phase microextraction (SPME). The parameters that can affect the SPME process were optimised: the type of fibre, the exposure time of the fibre, the temperature of extraction, the pH and ionic strength. The PDMS fibre was the most polyvalent for the extractions of the different pesticides studied.

Samples were analysed by gas chromatography coupled with ion-trap tandem mass-spectrometry (GC-ITD-MS/MS). Quantification of rainwater samples was performed by comparing the samples with standards. The detection limit for iprodione was 100 ng/L.

II. RESULTS AND DISCUSSION

Findings

Iprodione was only detected in two out of 78 the rainwater samples collected. In the samples taken between 4th March 2002 and 4th January 2003 (n=30), iprodione was only detected once in a sample from Strasbourg at a concentration below the quantification limit. In the rainwater collected between 4th January 2003 and 20th July 2003 (n=9), iprodione was detected once in a sample from Erstein, but again the concentration was below the quatification limit.

III. CONCLUSION

Method evaluation showed that the concentrations of 18 out of 20 selected pesticides could be determined using a combination of SPME and GC-ITD-MS/MS. Iprodione was detected once in rainwater samples from Strasbourg and Erstein, respectively, at concentrations below the quantification limit.

Iprodione

DOCUMENT M-CA, Section 8

ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Compiled by:

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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 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

CA 8.1 Effects on birds and other terrestrial vertebrates

Studies conducted for use in the risk assessments for birds and mammals

Four new field studies were conducted to support evaluation of the potential risks from iprodione to birds and mammals. In conjunction with these studies a method validation had to be done and reported. Also, six further reports on the evaluation of the results as obtained in the field studies were issued. The summaries of all these 11 aforementioned studies and documents (for an overview see Table 8.1-1) are given further down. One field effect study on small mammals' populations (BASF DocID 2014/1000061) is being conducted in 2014 in agreement with ANSES the draft report of which will only be submitted by September 2014, hence a summary is not yet available.

Beside those studies and reports conducted using or being based on the representative iprodione formulation, further studies are used in the risk assessments for birds and mammals. Since these are generic ones, the summaries of them are found in Document M-CP at the beginning of chapter 10.1.

Table 8.1-1: Overview of studies / reports / documents whose summaries are given further down

Data point	Author(s)	Year	Title Reference (BASF DocID)	Included in Application of Oct 2013 [1] [yes/no]
CA 8.1/1	Martin T.	2014	Study on the residue behavior of Iprodione (BAS 610 F) on pea (young plants) after the application of BAS 610 06 F under field conditions in Germany, Netherlands, Italy and Spain, 2013 2013/1308430	yes
CA 8.1/2	Moreno S., Galvez O.	2014	Study on the residue behaviour of Iprodione (BAS 610 F) on wheat (young plants) after treatment with BAS 610 06 F under field conditions in North and South Europe, season 2012-2013 2013/1078071	yes
CA 8.1/3	Szegedi K.	2014	Dissipation of BAS 610 F - Iprodione on young plants (wheat and peas) - Kinetic evaluation of trials conducted in the Southern Zone of Europe 2014/1046499	no [2]
CA 8.1/4	Szegedi K.	2014	Dissipation of BAS 610 F - Iprodione on young plants (wheat and peas) - Kinetic evaluation of trials conducted in the Northern Zone of Europe 2014/1046500	no [2]

Data point	Author(s)	Year	Title Reference (BASF DocID)	Included in Application of Oct 2013 [1] [yes/no]
CA 8.1/5	Henkes K., Pflanz D.	2014	Iprodione in BAS 610 06 F - Iprodione residues on arthropods (foliage- and ground dwellers) following spray application in annual field crops (oil seed rape) in Spain 2013/1096101	yes
CA 8.1/6	Henkes K., Pflanz D.	2014	Iprodione in BAS 610 06 F - Iprodione residues on arthropods (foliage- and ground-dwellers) following spray application in annual field crops (oil seed rape) in Germany 2013/1096100	yes
CA 8.1/7	Kuhn T.	2014	Development and validation of an analytical method for the determination of Iprodione and its metabolite Reg.No. 5079628 (formerly RP32490) in arthropods 2013/1311888	yes
CA 8.1/8	Shbaita H.	2014	Calculation of DT_{50} dissipation times of BAS 610 F – Iprodione in arthropods after spray application on oil seed rape in Spain 2014/1035860	no [3]
CA 8.1/9	Shbaita H.	2014	Calculation of dissipation times (DT_{50}) for BAS 610 F - Iprodione in arthropods after spray application on oil seed rape in Germany 2014/1035859	no [3]
CA 8.1/10	Henkes K.	2014	Time-weighted average (TWA) calculation based on Iprodione residues in ground-dwelling arthropods collected in Spain an in Henkes *et al.* (2014) 2014/1017045	no [3]
CA 8.1/11	Henkes K.	2014	Time-weighted average (TWA) calculation based on Iprodione residues in ground-dwelling arthropods collected in Germany an in Henkes *et al.* (2014) 2014/1017046	no [3]
CA 8.1/12	Anonymous	2014	Long-term field study to assess potential impact of an iprodione foliar spray formulation (BAS 610 06 F) on small mammal populations in Central Europe (Germany) 2014/1000061 Summary not yet available, study being conducted in 2014	yes

1 BASF DocID 2013/1328269
2 This report could not be included in the Application as its content and hence compilation is entirely based on the field results on residue behaviour of iprodione in plants (see CA 8.1/1, CA 8.1/2) which were not available at the time of the Application submission.
3 This report could not be included in the Application as its content and hence compilation is entirely based on the field results on residue behaviour of iprodione in arthropods (see CA 8.1/5, CA 8.1/6) which were not available at the time of the Application submission.

Studies conducted for use in the risk assessments for birds and mammals

Report:	CA 8.1/1 Martin T., 2014a Study on the residue behavior of Iprodione (BAS 610 F) on pea (young plants) after the application of BAS 610 06 F under field conditions in Germany, Netherlands, Italy and Spain, 2013 2013/1308430
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, EEC 7029/VI/95 rev. 5 Appendix B (July 22 1997), EEC 7525/VI/95 rev. 9 (March 2011)
GLP:	yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid Spain)

The objective of the study was to determine the magnitude of residues of iprodione (BAS 610 F) in young pea plants after single application of BAS 610 06 F at application rates of 750 g a.s./ha and 1 125 g a.s./ha.

I. MATERIAL AND METHODS

Study site

During the 2013 growing season a total of eight trials were conducted on four sites in representative pea growing areas in Germany, The Netherlands, Spain and Italy respectively, in order to determine the residue level of iprodione (BAS 610 F) after application of BAS 610 06 F in young pea plants.

Information on test item, application, sampling and residue determination

Each site consisted of one untreated (plot 1) and two treated plots (plot 2 and plot 3). BAS 610 06 F (75% of iprodione, WG) was applied once on plot 2 at a rate equivalent to 750 g/ha of iprodione (1.0 kg of formulated product/ha) and once on plot 3 at a rate equivalent to 1 125 g/ha of iprodione (1.5 kg of formulated product/ha). The spray volume used was 200 L/ha and the application was done at BBCH 12-13. Specimens of whole plant without roots were collected on plot 1 at the day of the application and 5 and 14 days thereafter. Specimens of whole plant without roots were collected on plot 2 and plot 3, 1 hour after the application and 1, 2, 3, 4, 5, 7, 10, 12 and 14 days thereafter. All specimens were analyzed for BAS 610 F using BASF method No. L0180/01 which has a limit of quantitation of 0.01 mg/kg. The results of procedural recovery experiments are shown in the following table.

II. RESULTS

Summary of residues of BAS 610 F in peas

Sampling No.	Plot	Portion analyzed	DALA[1)]	Growth stage	n	Range of BAS 610 F residues [mg/kg]
1	2	Whole plant (no roots)	0 [2)]	12-13	4	24 – 50
	3					32 – 62
2	2		1	12-13	4	4.7 – 34
	3					13 – 66
3	2		2	12-14	4	5.1 – 29
	3					11 – 46
4	2		3	12-14	4	3.1 – 23
	3					7.4 – 47
5	2		4	13-15	4	3.4 - 20
	3					7.8 – 33
6	2		5	13-15	4	1.6 – 21
	3					5.5 – 30
7	2		7	13-16	4	1.5 – 15
	3					4.2 – 25
8	2		10	14-17	4	0.9 – 11
	3					2.1 – 11
9	2		12	15-17	4	0.53 – 5.5
	3					1.7 – 7.3
10	2		14	15-18	4	0.47 – 5.9
	3					0.95 – 6.6

1 Days after last application
2 1h after last application (1 HALA)

III. CONCLUSION

In young pea plants the residues of BAS 610 F ranged between 24 mg/kg and 50 mg/kg directly after the application in plot 2 with a nominal application rate of 750 g a.s./ha. Afterwards the residues decreased to a range of 0.47 mg/kg and 5.9 mg/kg at day 14 after the application. In plot 3 with a nominal application rate of 1 125 g a.s./ha the residues of BAS 610 F ranged between 32 mg/kg and 62 mg/kg directly after the last application. Afterwards the residues decreased continuously to a range of 0.95 mg/kg and 6.6 mg/kg at day 14 after the application. No residues of iprodione (BAS 610 F) at or above the limit of quantitation (0.01 mg/kg) were detected in the untreated specimens of this study excepting for sample L1206850001 (0.013 mg/kg). This result in the untreated specimen can be explained by slight contamination that could have happened in the field, in the specimen management or in the laboratory. The small residue amount found in this untreated specimen doesn´t affect the residue results of the study.

Report:	CA 8.1/2 Moreno S.,Galvez O., 2014a Study on the residue behaviour of Iprodione (BAS 610 F) on wheat (young plants) after treatment with BAS 610 06 F under field conditions in North and South Europe, season 2012-2013 2013/1078071
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, EEC 7029/VI/95 rev. 5 Appendix B, EEC 7525/VI/95 rev. 9 (March 2011)
GLP:	yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid, Spain)

The objective of the study was to determine the magnitude of residues of iprodione (BAS 610 F) in young wheat plants after single application of BAS 610 06 F at application rates of 750 g a.s./ha and 1 125 g a.s./ha.

I. MATERIAL AND METHODS

Study site

During the 2013 growing season a total of eight trials were conducted on four sites in representative wheat growing areas in Germany, The Netherlands, Spain and Italy respectively, in order to determine the residue level of iprodione (BAS 610 F) after application of BAS 610 06 F in young wheat plants.

Information on test item, application, sampling and residue determination

Each site consisted of one untreated (plot 1) and two treated plots (plot 2 and plot 3). The test item BAS 610 06 F, a water dispersible granule (WG), was foliar applied on wheat one time (BBCH 12-13) at two different rates (750 g a.s./ha and 1 125 g a.s./ha nominal for plots 2 and 3 respectively) with a water volume of 200 l/ha. Untreated wheat (whole plant (no roots)) specimens were sampled at 1 HALA (hours after last application) and at 5 and 14 DALA (days after last application). Treated wheat (whole plant (no roots)) specimens were sampled at 1 HALA (hours after last application) and at 1, 2, 3, 4, 5, 7, 10, 12 and 14 DALA (days after last application). Untreated plots were sampled before the treated plots. All wheat specimens were analysed for BAS 610 F using BASF method L0180/01, which has a limit of quantitation (LOQ) of 0.01 mg/kg. The results of procedural recovery experiments are shown in the following table.

II. RESULTS

Summary of residues of BAS 610 F in wheat

Sampling No.	Plot	Portion analyzed	DALA[1)]	Growth stage	n	Range of BAS 610 F residues [mg/kg]
1	2	Whole plant (no roots)	0 [2)]	12-13	4	31-48
	3					39-106
2	2		1	12-13	4	25-53
	3					37-102
3	2		2	12-14	4	25-33
	3					27-80
4	2		3	12-14	4	19-44
	3					24-75
5	2		4	12-14	4	17-41
	3					21-64
6	2		5	13-14	4	14-50
	3					12-63
7	2		7	13-15	4	3.0-24
	3					7.3-50
8	2		10	13-21	4	3.3-22
	3					4.2-39
9	2		12	13-21	4	1.6-15
	3					1.5-28
10	2		14	13-23	4	0.47-12
	3					0.87-19

1 DALA=Days after last application
2 1 HALA = 1h after last application

III. CONCLUSION

In young wheat plants the residues of BAS 610 F ranged between 31 mg/kg and 48 mg/kg directly after the application in plot 2 with a nominal application rate of 750 g a.s./ha. The residues at one day after the application measured between 25 mg/kg and 53 mg/kg. Afterwards the residues decreased continuously to a range of 0.47 mg/kg and 12 mg/kg at day 14 after application. In plot 3 with a nominal application rate of 1 125 g a.s./ha the residues of BAS 610 F ranged between 39 mg/kg and 106 mg/kg directly after application. Afterwards the residues decreased continuously to a range of 0.87 mg/kg and 19 mg/kg at day 14 after the application.

Residues of BAS 610 F were below 0.010 mg/kg in all control specimens except for sample L1206870001.

Report: CA 8.1/3
Szegedi K., 2014a
Dissipation of BAS 610 F - iprodione on young plants (wheat and peas) - Kinetic evaluation of trials conducted in the Southern Zone of Europe 2014/1046499
Guidelines: none
GLP: no

This modelling report provides kinetic analysis and estimation of dissipation times (DT50, DT90 values) for iprodione in young plants.

I. MATERIAL AND METHODS

Kinetic analysis and calculations of DT50 and DT90 values were performed considering the recommendations of the FOCUS Kinetics workgroup for the derivation of modeling endpoints. The analysis was done by non-linear regression methods using the software package KinGUI v1.2. The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS guidance. As goodness-of-fit measures, the X2 minimum error level is provided.

II. RESULTS

DT_{50} values of iprodione in young wheat and pea plants in Southern Europe based on kinetic modeling

Plant	Trial	Plot[1)]	DT_{50} [d]	DT_{90} [d]	Kinetic model	χ^2 error	DT_{50} (modeling) [d]
Peas	L120685	P2	3.55	11.79	SFO [3)]	11.43	3.55
	L120685	P3	3.20	10.63	SFO [3)]	13.65	3.20
	L120686	P2	3.12	10.36	SFO [3)]	16.56	3.12
	L120686	P3	3.80	12.62	SFO [3)]	13.49	3.80
Wheat	L120689	P2	10.69	35.51	SFO [3)]	11.2	10.69
	L120689	P3	6.36	21.13	SFO [3)]	13.0	6.36
	L120690	P2	4.56 [2)]	15.15	SFO [3)]	35.8	4.56-
	L120690	P3	3.77	12.52	SFO [3)]	7.5	3.77

1 Treatment P2 with nominally 0.750 kg a.s./ha, treatment P3 with nominally 1.125 kg a.s./ha of iprodione
2 Conservative parameter
3 Single first-order kinetics

III. CONCLUSION

The decline of iprodione residues on young plants was well described by this first order kinetics. The DT50 of iprodione in young pea plants ranges between 3.12 and 3.80 days. The DT50 of iprodione in young wheat plants ranges between 3.77 and 10.69 days.

Report: CA 8.1/4
Szegedi K., 2014b
Dissipation of BAS 610 F - Iprodione on young plants (wheat and peas) - Kinetic evaluation of trials conducted in the Northern Zone of Europe 2014/1046500
Guidelines: none
GLP: no

This modelling report provides kinetic analysis and estimation of dissipation times (DT50, DT90 values) for iprodione in young plants.

I. MATERIAL AND METHODS

Kinetic analysis and calculations of DT50 and DT90 values were performed considering the recommendations of the FOCUS Kinetics workgroup for the derivation of modeling endpoints. The analysis was done by non-linear regression methods using the software package KinGUI v1.2. The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS guidance. As goodness-of-fit measures, the X2 minimum error level is provided.

II. RESULTS

DT_{50} values of iprodione in young wheat and pea plants in Northern Europe based on kinetic modeling

Plant	Trial	Plot[1)]	DT_{50} [d]	DT_{90} [d]	Kinetic model	χ^2 error	DT_{50} (modeling) [d]
Pea	L120683	P2	0.15	2.76	FOMC [2)]	11.6	0.83
	L120683	P3	0.87	9.28	FOMC [2)]	9.35	2.80
	L120684	P2	5.51	18.3	SFO [3)]	7.2	5.51
	L120684	P3	4.06	13.49	SFO [3)]	14.03	4.06
Wheat	L120687	P2	4.11	13.65	SFO [3)]	10.97	4.11
	L120687	P3	3.24	10.76	SFO [3)]	9.78	3.24
	L120088	P2	2.97	9.87	SFO [3)]	9.06	2.97
	L120088	P3	2.60	8.64	SFO [3)]	8.55	2.60

1 Treatment P2 with nominally 750 g/ha, treatment P3 with nominally 1125 g/ha of iprodione
2 First-order multi-compartment kinetics
3 Single first-order kinetics

III. CONCLUSION

The decline of iprodione residues on young wheat plants was well described by this first order kinetics. The DT50 of iprodione in young pea plants ranges between 0.83 and 4.06. The DT50 of iprodione in young wheat plants ranges between 2.60 and 4.11 days.

Report:	CA 8.1/5 Henkes K.,Pflanz D., 2014a Iprodione in BAS 610 06 F - Iprodione residues on arthropods (foliage- and ground dwellers) following spray application in annual field crops (oil seed rape) in Spain 2013/1096101
Guidelines:	none
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

The aim of this study was to determine residues levels of iprodione (BAS 610 F) and its metabolite Reg. No. 5079628 (RP 32490) in ground- and foliage-dwelling arthropods in an oilseed rape field in Spain, after spray applications of the end-use product BAS 610 06 F. The results from the current study are expected to form a basis for assessing the risk of BAS 610 06 F to insectivorous and omnivorous birds and mammals.

I. Materials and methods

Study site

The field study was conducted in a summer oilseed rape field situated in the region of Albacete, which is a typical oilseed rape growing area in Spain. The study field represented a typical oilseed rape field in the region as to both size (2.8 ha) and basic structure (i.e. distance between plant rows). Within the study field three study plots (=replicates) were set up with areas of 0.85 ha or 1.10 ha.

Test item and application

The test item BAS 610 06 F (containing nominal 75% of the active substance iprodione, WG formulation) was applied twice on 09 May 2013 and 17 June 2013 in accordance with Good Agricultural Practice and Good Laboratory Practice at a nominal application rate of 1125 g a.s./ha (corresponding to 1.5 kg BAS 610 06 F/ha) in a spray volume of 300 L water/ha at oilseed rape BBCH growth stage 12 (first application) and 62 (second application).The actual application rates for the first application was 1120 g a.s./ha in a spray volume of 299 L water/ha at study plot 1, 1089 g a.s./ha in a spray volume of 290 L water/ha at study plot 2 and 1084 g a.s./ha in a spray volume of 289 L/ha at study plot 3. The actual application rate for the second application was 1117 g a.s./ha in a spray volume of 298 L water/ha at study plot 1, 1113 g a.s./ha in a spray volume of 297 L water/ha at study plot 2 and 1095 g a.s./ha in a spray volume of 292 L/ha at study plot 3.

Arthropod sampling

Samples of natural populations of ground-dwelling arthropods were collected for residue analysis by pitfall trapping, whereas foliage-dwelling arthropods were collected by inventory spraying (using a knock-down insecticide AquaPy®: natural pyrethrum 30 g a.s./L and Piperonylbutoxid, 150 g/L; spraying under Non-GLP) The pitfall traps were activated (opened) approx. 24 h before sampling. Over a period of 55 days, altogether 20 sampling events took place for ground-dwelling arthropods with samples taken on each of the three study plots separately. A pre-sampling was conducted one day before first application, thereafter further samples were collected at DAFT (day after first treatment) 1, 2, 3, 4, 5, 7, 9, 11, 14 and DAST (day after second treatment) -3, 1, 2, 3, 4, 5, 7, 9, 11, 14 corresponding to consecutive days 36, 40, 41, 42, 43, 44, 46, 48, 50 and 53 when related to the first application event only. At each sampling event, per study plot, arthropods were collected from all individual pitfall traps and pooled to provide a single sample per study plot. A defined area of 20 m² (corresponding to 100 gutters) oilseed rape was treated with AquaPy® to obtain the minimum foliage-dwelling arthropod matrix of 3 g. All foliage-dwellers recovered from the gutters (positioned between the rape plants) at each sampling event on each study plot were pooled to obtain a single sample for each study plot. Inventory spraying, hence sampling of foliage-dwelling arthropods was done 12 times. The samples were collected at DAST -3, -2, 0 (approximately 5 h after application), 1, 2, 3, 4, 5, 7, 9, 11 and 14 corresponding to consecutive days 36, 37, 39, 40, 41, 42, 43, 44, 46, 48, 50, and 53 when related to the first application event only. After determination of the main taxonomic orders the arthropod samples (ground-dwellers and foliage-dwellers) were weighed and stored at a temperature of at least -18°C until residue analysis.

Residue analysis

The arthropod specimens were analysed for iprodione and its metabolite Reg. No. 5079628 by LC/MS/MS. The limit of quantification (LOQ) was 0.01 mg/kg per analyte.

Calculation

The initial and maximum concentrations of iprodione and its metabolite Reg. No. 5079628 in ground-dwelling arthropods, as well as the 90th percentiles, were calculated based on the arithmetic mean of three replicates (n=3). Additionally, the initial and maximum concentrations of iprodione and its metabolite Reg. No. 5079628 in foliage-dwelling arthropods, as well as the 90th percentiles, were reported on the arithmetic mean of three replicates (n=3). For ground-dwelling arthropods two initial and maximum concentrations per analyte, as well as two maximum 90th percentiles per analyte were calculated, the ones for the period from the first to the second application and the ones after the second application.

II. Results

Residues of iprodione in ground-dwelling arthropods

Residues between first and second application of nominal 1125 g iprodione/ha				
	Iprodione		Metabolite Reg. No. 5079628	
	Residues [mg a.s./kg f.w.]	DAFT	Residues [mg a.s./kg f.w.]	DAFT
Initial mean measured concentration [1)]	11.00	1	2.55	1
Maximum mean measured concentration [1)]	19.27	4	6.02	4
Maximum 90th percentile [2)]	27.20	4	8.97	3
Residues after second application of nominal 1125 g iprodione/ha				
	Iprodione		Metabolite Reg. No. 5079628	
	Residues [mg a.s./kg f.w.]	DAST	Residues [mg a.s./kg f.w.]	DAST
Initial mean measured concentration [1)]	10.89	1	2.89	1.
Maximum mean measured concentration [1)]	11.69	2	2.99	3
Maximum 90th percentile [2)]	15.92	2	3.33	2

1 arithmetic mean, n=3
2 based on three replicates (n=3)
DAFT=Day after first treatment
DAST=Day after second treatment
Limit of Quantification (LOQ) for iprodione and its metabolite Reg. No. 5079628 was 0.01 mg/kg.

Residues of iprodione in foliage-dwelling arthropods

Residues after second application of nominal 1125 g iprodione/ha				
	Iprodione		Metabolite Reg. No. 5079628	
	Residues [mg a.s./kg f.w.]	DAST	Residues [mg a.s./kg f.w.]	DAST
Initial mean measured concentration [1)]	50.60	0	1.89	0
Maximum mean measured concentration [1)]	50.60	0	5.02	2
Maximum 90th percentile [2)]	60.70	0	6.10	4

1 arithmetic mean, n=3
2 based on three replicates (n=3)
DAST=Day after second treatment
Limit of Quantification (LOQ) for iprodione and its metabolite Reg. No. 5079628 was 0.01 mg/kg.

III. Conclusion

The study provides field data on the magnitude of initial residue levels and the subsequent time course of residues of iprodione and its metabolite Reg. No. 5079628 in ground- and foliage-dwelling arthropods. The maximum residue concentrations of iprodione (arithmetic mean, n=3) in ground-dwelling arthropods after the first application were 19.27 ± 9.57 mg a.s./kg f.w. (DAFT 4) and after the second application 11.69 ± 5.14 mg a.s./kg f.w (DAST 2). The maximum 90th percentiles of iprodione residue concentrations (n=3) after the first application were 27.20 mg a.s./kg f.w. (DAFT 4) and after the second application 15.92 mg a.s./kg f.w (DAST 2).
The maximum residue concentrations of the metabolite Reg. No. 5079628 (arithmetic mean, n=3) in ground-dwelling arthropods after the first application were 6.02 ± 1.39 mg a.s./kg f.w. (DAFT 4) and after the second application 2.99 ± 0.23 mg (DAST 3). The maximum 90th percentiles of metabolite Reg. No. 5079628 residue concentrations (n=3) after the first application were 8.97 mg a.s./kg f.w. (DAFT 3) and after the second application 3.33 mg a.s./kg f.w (DAST 2).
The maximum residue concentration of iprodione (arithmetic mean, n=3) in foliage-dwelling arthropods after the second application was 50.60 ± 12.15 mg a.s./kg f.w. (DAST 0). The maximum 90th percentile of iprodione residue concentrations (n=3) after the second application was 60.70 mg a.s./kg f.w. (DAST 0).
The maximum residue concentration of the metabolite Reg. No. 5079628 in foliage-dwelling arthropods after the second application was 5.02 ± 0.28 mg a.s./kg f.w. (DAST 2). The maximum 90th percentile of metabolite Reg. No. 5079628 residue concentrations (n=3) after the second application was 6.10 mg a.s./kg f.w. (DAST 4). These data provide a reliable basis for use in higher tier risk assessments for insectivorous or omnivorous birds and mammals.

Report:	CA 8.1/6 Henkes K.,Pflanz D., 2014a Iprodione in BAS 610 06 F - Iprodione residues on arthropods (foliage- and ground-dwellers) following spray application in annual field crops (oil seed rape) in Germany 2013/1096100
Guidelines:	none
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

The aim of this study was to determine residues levels of iprodione (BAS 610 F) and its metabolite Reg. No. 5079628 (RP 32490) in ground- and foliage-dwelling arthropods in oilseed rape fields, after spray applications of the end-use product BAS 610 06 F. The results from the present study are expected to form a basis for assessing the risk of BAS 610 06 F to insectivorous and omnivorous birds and mammals.

I. Materials and methods

Study site

The field study was conducted in three summer oilseed rape fields situated close to the village of Brensbach (in the federal state Hessen) situated in a typical oilseed rape growing area in Southern Germany. The study fields represented typical oilseed rape fields in the region as to both field size (1.43-3.95 ha) and basic structure (i.e. distance between plant rows). Within each study field one study plot (=replicates) was set up with a surface of approximately 1.0 ha.

Test item and application

The test item BAS 610 06 F (containing nominal 75% of the active substance: iprodione, WG formulation) was applied twice on 11 May 2013 and 26 June 2013 in accordance with Good Agricultural Practice and Good Laboratory Practice at a nominal application rate of 1125 g a.s./ha (corresponding to 1.5 kg/ha BAS 610 06 F) in a spray volume of 250 L water/ha at oilseed rape BBCH growth stage 11 (at first application) and 59 to 60 (at second application). The actual application rate for the first application was 1117 g a.s./ha in a spray volume of 290.4 L water/ha at study plot 1, 1141 g a.s./ha in a spray volume of 271.3 L water/ha at study plot 2 and 1139 g a.s./ha in a spray volume of 317.7 L/ha at study plot 3. The actual application rate for the second application was 1100 g a.s./ha in a spray volume of 286.3 L water/ha at study plot 1, 1109 g a.s./ha in a spray volume of 263.6 L water/ha at study plot 2 and 1117 g a.s./ha in a spray volume of 311.7 L/ha at study plot 3.

Arthropod sampling

Samples of natural populations of ground-dwelling arthropods were collected for residue analysis by pitfall trapping, whereas foliage-dwelling arthropods were collected by inventory spraying (using a knock-down insecticide AquaPy®: natural pyrethrum 30 g a.s./L and Piperonylbutoxid, 150 g/L; spraying under Non-GLP). The pitfall traps were activated (opened) approx. 24 h before sampling. Over a period of 59 days, altogether 17 sampling events took place for ground-dwelling arthropods with samples taken on each of the three study plots separately. A pre-sampling was conducted seven days before the first application (DAFT -7), thereafter further samples were collected at DAFT (day after first treatment) -1, 1, 2, 3, 4, 5, 7, 9, 11, 14 and DAST (day after second treatment) -1, 1, 2, 3, 4, 5, corresponding to consecutive days 45, 47, 48, 49, 50 and 51 when related to the first application event only (Table A 4). At each sampling event, per study plot, arthropods were collected from all individual pitfall traps and pooled to provide a single sample per study plot. A defined surface of 20 m² (corresponding to 100 gutters) oilseed rape was treated with AquaPy® to obtain the minimum foliage-dwelling arthropod matrix of 3 g. All foliage-dwellers recovered from the gutters (positioned between the rape plants) at each sampling event on each study plot were pooled to obtain a single sample for each study plot. Inventory spraying, hence sampling of foliage-dwelling arthropods was done 11 times. The samples were collected before the second application at DAST -0 (approximately 8 hours before application) and after the second application at DAST +0 (approximately 4 hours after the first application), 2, 3, 4, 5, 6, 8, 9, 11 and 14 corresponding to consecutive days 46 (approximately 3.5 hours after the second application), 48, 49, 50, 51, 52, 54, 55, 57 and 60 when related to the first application event only. After determination of the main taxonomic orders the arthropod samples (ground-dwellers and foliage-dwellers) were weighed and stored at a temperature of at least -18°C until residue analysis was conducted.

Residue analysis

The arthropod specimens were analysed for iprodione and its metabolite Reg. No. 5079628 by LC/MS/MS. The limit of quantification (LOQ) was 0.01 mg/kg per analyte.

Calculation

The initial and maximum concentrations of iprodione and its metabolite Reg. No. 5079628 in ground-dwelling arthropods, as well as the 90th percentiles, were calculated based on the arithmetic mean of three replicates (n=3). Additionally, the initial and maximum concentrations of iprodione and its metabolite Reg. No. 5079628 in foliage-dwelling arthropods, as well as the 90th percentiles, were reported based on the arithmetic mean of three replicates (n=3). For ground-dwelling arthropods two initial and maximum concentrations per analyte, as well as two maximum 90th percentiles per analyte were calculated, the ones for the period from the first to the second application and the ones after the second application.

II. Results

Residues of iprodione in ground-dwelling arthropods

Residues between first and second application of nominal 1125 g iprodione/ha				
	Iprodione		Metabolite Reg. No. 5079628	
	Residues [mg a.s./kg f.w.]	DAFT	Residues [mg a.s./kg f.w.]	DAFT
Initial mean measured concentration 1)	1.93	1	0.45	1
Maximum mean measured concentration 1)	1.93	1	0.74	14
Maximum 90th percentile 2)	2.38	4	1.00	14
Residues after second application of nominal 1125 g iprodione/ha				
	Iprodione		Metabolite Reg. No. 5079628	
	Residues [mg a.s./kg f.w.]	DAST	Residues [mg a.s./kg f.w.]	DAST
Initial mean measured concentration 1)	1.25	1	0.82	1
Maximum mean measured concentration 1)	1.25	1	1.33	2
Maximum 90th percentile 2)	1.74	1	1.56	2

1 arithmetic mean, n=3
2 based on three replicates (n=3)
DAFT=Day after first treatment
DAST=Day after second treatment
Limit of Quantification (LOQ) for iprodione and its metabolite Reg. No. 5079628 was 0.01 mg/kg.

Residues of iprodione in foliage-dwelling arthropods

Residues after second application of nominal 1125 g iprodione/ha				
	Iprodione		Metabolite Reg. No. 5079628	
	Residues [mg a.s./kg f.w.]	DAST	Residues [mg a.s./kg f.w.]	DAST
Initial mean measured concentration 1)	65.33	+0	0.67	+0
Maximum mean measured concentration 1)	65.33	+0	2.36	3
Maximum 90th percentile 2)	74.40	+0	2.80	3

1 arithmetic mean, n=3
2 based on three replicates (n=3)
DAST=Day after second treatment
Limit of Quantification (LOQ) for iprodione and its metabolite Reg. No. 5079628 was 0.01 mg/kg.

III. Conclusion

The study provides field data on the magnitude of initial residue levels and the subsequent time course of residues of iprodione and its metabolite Reg. No. 5079628 in ground- and foliage-dwelling arthropods. The maximum residue concentrations of iprodione (arithmetic mean, n=3) in ground-dwelling arthropods after the first application were 1.93 ± 0.24 mg a.s./kg f.w. (DAFT 1) and after the second application 1.25 ± 0.60 mg a.s./kg f.w (DAST 1). The maximum 90th percentiles of iprodione residue concentrations (n=3) after the first application were 2.38 mg a.s./kg f.w. (DAFT 4) and after the second application 1.74 mg a.s./kg f.w (DAST 1).
The maximum residue concentrations of the metabolite Reg. No. 5079628 (arithmetic mean, n=3) in ground-dwelling arthropods after the first application were 0.74 ± 0.32 mg a.s./kg f.w. (DAFT 14) and after the second application 1.33 ± 0.27 mg (DAST 2). The maximum 90th percentiles of metabolite Reg. No. 5079628 residue concentrations (n=3) after the first application were 1.00 mg a.s./kg f.w. (DAFT 14) and after the second application 1.56 mg a.s./kg f.w (DAST 2).
The maximum residue concentrations of iprodione (arithmetic mean, n=3) in foliage-dwelling arthropods after the second application was 65.33 ± 12.39 mg a.s./kg f.w. (DAST +0). The maximum 90th percentile of iprodione residue concentrations (n=3) after the second application was 74.40 mg a.s./kg f.w. (DAST +0).
The maximum residue concentrations of the metabolite Reg. No. 5079628 in foliage-dwelling arthropods after the second application was 2.36 ± 0.66 mg a.s./kg f.w. (DAST 3). The maximum 90th percentile of metabolite Reg. No. 5079628 residue concentrations (n=3) after the second application was 2.80 mg a.s./kg f.w. (DAST 3).
These data provide a reliable basis for use in higher tier risk assessments for insectivorous or omnivorous birds and mammals.

Report: CA 8.1/7
Kuhn T., 2014a
Development and validation of an analytical method for the determination of Iprodione and its metabolite Reg.No. 5079628 (formerly RP32490) in arthropods
2013/1311888
Guidelines: SANCO/3029/99 rev. 4 (11 July 2000)
GLP: yes
(certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

The objective was to develop and to validate an analytical method for iprodione and its metabolite Reg. No. 5079628 in arthropods at a limit of quantitation (LOQ) of 0.01 mg/kg.

I. METHOD

Iprodione and its metabolite Reg. No. 5079628 are extracted from arthropods with acetone. An aliquot of the acetone extract is concentrated to dryness and re-constituted in acetonitrile / water (50/50, v/v) containing 0.1 % formic acid. Final determination of the analytes is achieved by LC-MS/MS, using MRM transitions for quantitation and confirmation. The limit of quantification (LOQ) of the method is 0.01 mg/kg.

II. RESULTS

For method validation, arthropods were fortified (5 replicates per fortification level) at 0.01 mg/kg (LOQ) and at 0.10 mg/kg (10xLOQ) with solutions containing both analytes. Additionally two specimens were kept untreated as blank controls to show that no significant LC-MS/MS signal interference was observed. Residues in all blank control specimens were below 30 % of the LOQ. The average recoveries for the ion transitions monitored were within the acceptable range of 70 % to 110 % with relative standard deviations (RSD) ≤ 20 %. The summary of the method validation results is given in the table below.

Summary of Recovery Results

Fortification Level	Analyte	Iprodione		Reg. No. 5079628	
		330 m/z ->	332 m/z ->	288 m/z ->	290 m/z ->
mg/kg	Recoveries	245 m/z	247 m/z	245 m/z	247 m/z
0.01	Average	99%	100%	91%	85%
	RSD	3%	3%	6%	6%
	n	5	5	5	5
0.10	Average	103%	100%	87%	89%
	RSD	2%	1%	5%	3%
	n	5	5	5	5
Overall	Average	101%	100%	89%	87%
	RSD	3%	2%	6%	5%
	n	10	10	10	10

III. CONCLUSION

PTRL Europe successfully performed the method validation for the determination of residues of the iprodione and its metabolite Reg. No. 5079628 in arthropods at a limit of quantitation (LOQ) of 0.01 mg/kg. The limit of detection (LOD) was set to 0.003 mg/kg. The method fulfils the requirements of SANCO/3029/99 rev. 4, 11/07/2000.

Report: CA 8.1/8
Shbaita H., 2014a
Calculation of DT50 dissipation times of BAS 610 F – Iprodione in arthropods after spray application on oil seed rape in Spain
2014/1035860
Guidelines: none
GLP: no

Executive Summary

The residue decline of iprodione (BAS 610 F) in foliage-dwelling arthropods has been studied in a field trial (with several plots) in Spain during the growing season 2013. This modelling report provides kinetic analysis and estimation of the dissipation times (DT50 values) for iprodione for each field data set.

I. MATERIAL AND METHODS

Since no specific recommendation is available how to carry out the kinetic evaluation for the described experiments, guidance of the FOCUS workgroup on degradation kinetics was used in order to derive endpoints for modelling purposes. This means the selected DT50 values are suitable input parameter for models that require single first order (SFO) DT50 values or conservative substitutes, respectively. The software package KinGUI version 2 was used for parameter fitting. The visual assessments of the residual plots show that the residuals are randomly scattered around the zero line and that the fits are acceptable for all datasets. The estimated dissipation rate constants are significantly different from zero as indicated by low t-test values.

II. RESULTS

The DT50 values that are suitable for modeling purposes according to FOCUS and the respective statistical indices are presented in the following table.

Southern Europe: DT_{50} dissipation times for iprodione in foliage-dwelling arthropods and statistical indices

crop	Trial	Kinetic model	DT_{50} (SFO) [d]	P (t-test)	$\chi 2$ error [%]
Oilseed rape	Plot 1	SFO	1.92	0.0322	14.65
	Plot 2	SFO	2.37	0.0330	16.28
	Plot 3	SFO	2.64	<0.001	14.83

III. CONCLUSION

The DT50 values which are suitable for modeling purposes according to FOCUS range from 1.92 to 2.64 days.

Report: CA 8.1/9
Shbaita H., 2014b
Calculation of dissipation times (DT50) for BAS 610 F - Iprodione in arthropods after spray application on oil seed rape in Germany
2014/1035859
Guidelines: none
GLP: no

The residue decline of iprodione (BAS 610 F) in foliage-dwelling arthropods has been studied in multiple field trials in Germany during the growing season 2013. This modelling report provides kinetic analysis and estimation of the dissipation times (DT50 values) for iprodione for each field data set.

I. MATERIAL AND METHODS

Since no specific recommendation is available how to carry out the kinetic evaluation for the described experiments, guidance of the FOCUS workgroup on degradation kinetics was used in order to derive endpoints for modelling purposes. This means the selected DT50 values are suitable input parameter for models that require single first order (SFO) DT50 values or conservative substitutes, respectively. The software package KinGUI version 2 was used for parameter fitting. The visual assessments of the residual plots show that the residuals are randomly scattered around the zero line and that the fits are acceptable for all datasets. The estimated dissipation rate constants are significantly different from zero as indicated by low t-test values.

II. RESULTS

The DT50 values that are suitable for modeling purposes according to FOCUS and the respective statistical indices are presented in the following table.

Northern Europe: DT_{50} dissipation times for iprodione in foliage-dwelling arthropods and statistical indices

crop	Trial	Kinetic model	DT_{50} (SFO) [d]	P (t-test)	$\chi 2$ error [%]
Oilseed rape	Plot 1	SFO	2.60	<0.001	16.24
	Plot 2	SFO	1.97	<0.001	15.84
	Plot 3	SFO	1.94	<0.001	21.46

III. CONCLUSION

The DT50 values which are suitable for modeling purposes according to FOCUS range from 1.94 to 2.60 days.

Report:	CA 8.1/10 Henkes K., 2014b Time-weighted average (TWA) calculation based on Iprodione residues in ground-dwelling arthropods collected in Spain as in Henkes et al. (2014) 2014/1017045
Guidelines:	EFSA Guidance Document: Risk assessment for birds and mammals (2009), EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009
GLP:	no

This report aims at calculating a time-weighted average value (TWA) for concentrations of iprodione and its metabolite Reg. No. 5079628 in ground-dwelling arthropods collected in Spain as presented in Henkes et al. (2014, BASF DocID 2013/1096101). The result is expected to form a basis for future risk assessments for insectivorous or omnivorous birds and mammals in the frame of EU Regulation No 1107/2009 and EFSA/2009/1438.

I. Material and Methods

Field study

The calculation was based on field data collected in Spain as presented in Henkes et al. (2014, BASF DocID 2013/1096101). The respective field study had been conducted to measure residues in ground-dwelling arthropods after spray application of BAS 610 06 F in an oilseed rape field in Spain. The sugar beet study field was situated approx. 18 km west of Albacete, which is a typical oilseed rape growing area in south-eastern Spain. Within the study field, three study plots (=replicate) were set up. Study plot 1 had a size of 1.1 ha and study plot 2 and 3 had a size of 0.85 h. The test item BAS 610 06 F (750 g active substance iprodione/kg, WG formulation) was applied twice, namely on 09 May 2013 and 17 June 2013 on all three study plots separately in accordance with Good Agricultural Practice at a nominal application rate of 1125 g iprodione/ha (corresponding to 1.5 kg/ha BAS 610 06 F) in a spray volume of 300 L water/ha. Samples of the natural populations of ground-dwelling arthropods were collected for residue analysis by pitfall trapping over a period of 55 days subsequent to first application. The arthropod specimens were analysed for iprodione and its metabolite Reg. No. 5079628 by LC/MS/MS. The limit of quantitation was 0.01 mg/kg for both analytes.

TWA calculation

The time-weighted average is a measure which describes a concentration (e.g. residue concentration) averaged over a given time span (e.g. over 14 days). Graphically, the TWA is represented by the area under the curve (AUC) of the time series plot of a function.
Time-weighted average (TWA) values of iprodione concentrations and concentrations of its metabolite Reg. No. 5079628 in ground-dwelling arthropods were determined for a 14-day period after the first application (DAFT 1-14) and for a 14-day period after the second application (DAST 1-14).

II. Results

The TWAs of iprodione and its metabolite Reg. No. 5079628 in ground-dwelling arthropods for the 14-day sampling period after the first application (DAFT 1-14) of BAS 610 06 F were 7.59 mg a.s./kg fresh weight and 3.41 mg a.s./kg fresh weight, respectively. The TWAs of iprodione and its metabolite Reg. No. 5079628 in ground-dwelling arthropods for the 14-day sampling period after the second application (DAST 1-14) of BAS 610 06 F were 3.29 mg a.s./kg fresh weight and 1.26 mg a.s./kg fresh weight, respectively.

III. Conclusion

This report provides TWA values for iprodione residues and residues of its metabolite Reg. No. 5079628 in ground-dwelling arthropods collected in Spain. The calculation was based on field data as presented in Henkes et al. (2014, BASF DocID 2013/1096101). The TWAs of iprodione and its metabolite Reg. No. 5079628 in ground-dwelling arthropods for the sampling period of 14 days subsequent to the first application were 7.59 mg a.s./kg fresh weight and 3.41 mg a.s./kg fresh weight, respectively. Furthermore, the TWAs of iprodione and its metabolite Reg. No. 5079628 in ground-dwelling arthropods for the sampling period of 14 days subsequent to the second application were 3.29 mg a.s./kg fresh weight and 1.26 mg a.s./kg fresh weight, respectively. These values provide a reliable basis for use in higher tier risk assessments for insectivorous or omnivorous birds and mammals.

Report:	CA 8.1/11 Henkes K., 2014a Time-weighted average (TWA) calculation based on Iprodione residues in ground-dwelling arthropods collected in Germany an in Henkes et al. (2014) 2014/1017046
Guidelines:	EFSA Guidance Document: Risk assessment for birds and mammals (2009), EC 1107/2009 (14 June 2011)
GLP:	no

This report aims at calculating a time-weighted average value (TWA) for concentrations of iprodione and its metabolite Reg. No. 5079628 in ground-dwelling arthropods collected in Germany as presented in Henkes et al. (2014, BASF DocID 2013/1096100). The result is expected to form a basis for future risk assessments for insectivorous or omnivorous birds and mammals in the frame of EU Regulation No 1107/2009 and EFSA/2009/1438.

I. Material and Methods

Field study

The calculation was based on field data collected in Germany as presented in Henkes et al. (2014, BASF DocID 2013/1096100). The respective field study had been conducted to measure residues in ground-dwelling arthropods after spray application of BAS 610 06 F on three individual oilseed rape fields in Germany. The three oilseed rape study fields were situated close to the city Brensbach situated in the federal state Hessen, in Southern Germany, a typical area of oilseed rape cultivation in Europe. Within each field, one study plot (=replicate) was set up with an area of approx. 1.0 ha, respectively. The test item BAS 610 06 F (active substance: iprodione, WG formulation) was applied twice, namely on 1 May 2013 and 26 June 2013 in accordance with Good Agricultural Practice at a nominal application rate of 1125 g iprodione/ha (corresponding to 1.5 kg/ha BAS 610 06 F) in a spray volume of 250 L water/ha. Samples of natural populations of ground-dwelling arthropods were collected for residue analysis by pitfall trapping during a period of 51 days subsequent to the first application. The arthropod specimens were analysed for iprodione and its metabolite Reg. No. 5079628 by LC/MS/MS. The limit of quantitation was 0.01 mg/kg for both analytes.

TWA calculation

The time-weighted average is a measure which describes a concentration (e.g. residue concentration) averaged over a given time span (e.g. over 14 days). Graphically, the TWA is represented by the area under the curve (AUC) of the time series plot of a function.
Time-weighted average (TWA) values of iprodione concentrations and concentrations of its metabolite Reg. No. 5079628 in ground-dwelling arthropods were determined for a 14-day period after the first application (DAFT 1-14) and for a 5-day period after the second application (DAST 1-5).

II. Results

The TWAs of iprodione and its metabolite Reg. No. 5079628 in ground-dwelling arthropods for the 14-day sampling period after the first application (DAFT 1-14) of BAS 610 06 F were 1.19 mg a.s./kg fresh weight and 0.56 mg a.s./kg fresh weight, respectively. The TWAs of iprodione and its metabolite Reg. No. 5079628 in ground-dwelling arthropods for the 5-day sampling period after the second application (DAST 1-5) of BAS 610 06 F were 0.83 mg a.s./kg fresh weight and 1.06 mg a.s./kg fresh weight, respectively.

III. Conclusion

This report provides TWA values for iprodione residues and residues of its metabolite Reg. No. 5079628 in ground-dwelling arthropods collected in Germany. The calculation was based on field data as presented in Henkes et al. (2014, BASF DocID 2013/1096100). The TWAs of iprodione and its metabolite Reg. No. 5079628 in ground-dwelling arthropods for the sampling period of 14 days subsequent to the first application were 1.19 mg a.s./kg fresh weight and 0.56 mg a.s./kg fresh weight, respectively. Furthermore, the TWAs of iprodione and its metabolite Reg. No. 5079628 in ground-dwelling arthropods for the sampling period of 5 days subsequent to the second application were 0.83 mg a.s./kg fresh weight and 1.06 mg a.s./kg fresh weight, respectively. These values provide a reliable basis for use in higher tier risk assessments for insectivorous or omnivorous birds and mammals.

Report: CA 8.1/12
Anonymous, 2014a
Long-term field study to assess potential impact of an Iprodione foliar spray formulation (BAS 610 06 F) on small mammal populations in Central Europe (Germany)
2014/1000061
Guidelines:
GLP: no

This study is ongoing

A summary of this study is not yet available since the study is being conducted in 2014.

In accordance with the RMS France and the Co-RMS Belgium conduct of a long-term field-effect study was launched early in the year 2013 as a weight of evidence approach to determine potential impact of BAS 610 06 F on resident small mammal populations under field conditions. However as communicated to (June 17, 2013) and agreed by ANSES the small mammals field effect study had to be stopped in 2013. Despite all efforts done in the field, the number of trapped small mammals as a whole was too small (on 9 out of 17 fields, zero animals were trapped for 13 nights of trapping) and did not even increase in early summer (June) of that year. It became doubtful, whether the study would bring conclusive results in the end as exposure to sufficient animals would have been difficult to demonstrate hence interpretation of any results.

Repeat of the long-term field effect study in 2014 was accepted by ANSES and will be carried out. In accordance with the RMS, a full draft report will be provided to ANSES September 2014 for inclusion in the DRAR.

Information on Chapters MCA 8.1.1 and 8.1.2

In the context of chapters MCA 8.1.1 and 8.1.2, 'Effects on birds' and 'Effects on terrestrial vertebrates other than birds', Table 8.1-2 provides information on the EU-reviewed and agreed toxicity endpoints, as well as additional toxicity studies relevant for AIR3 for the active substance iprodione (BAS 610 F) for assessing the risk to birds and mammals.

Table 8.1-2: Summary of EU-reviewed and agreed, as well as additional toxicity studies relevant for AIR3 for the active substance iprodione (BAS 610 F) for assessing the risk to birds and mammals [1)]

Test system	Test species	Reference [Author, BASF DocID, Year]	EU-agreed
BIRDS			
Acute oral toxicity	*Colinus virginianus*	Culotta *et al.*, C021504, 1990	Yes (still valid for AIR 3 according to EFSA/2009/1438)
Short-term dietary toxicity	*Colinus virginianus*	Driscoll *et al.*, C021500, 1990	Yes (but no longer part of core data package according to EFSA/2009/1438)
	Anas platyrhynchos	Driscoll *et al.*, C021501, 1990	Yes (but no longer part of core data package according to EFSA/2009/1438)
Sub-chronic toxicity and reproduction	*Colinus virginianus*	Beavers and Fink, R014619, 1981	Yes (still valid for AIR 3 according to EFSA/2009/1438)
	Anas platyrhynchos	Beavers and Fink, C021503, 1980	Yes (still valid for AIR 3 according to EFSA/2009/1438)
MAMMALS			
Acute oral toxicity	Rat	Cummins, C020989, 1989	Yes (still valid for AIR 3 according to EFSA/2009/1438)
2-Generation reproductive toxicity	Rat	Henwood, C021572, 1991	Yes (still valid for AIR 3 according to EFSA/2009/1438)
		Schneider, 2013/1251918, 2013	No (new study)
Prenatal Development toxicity	Rat	Coquet, C022944, 1973	Yes (still valid for AIR 3 according to EFSA/2009/1438)
	Rat	Tesh, C023030, 1986	Yes (still valid for AIR 3 according to EFSA/2009/1438)
	Rat	Repetto-Larsay, C022658, 1997	Yes (still valid for AIR 3 according to EFSA/2009/1438)
	Rabbit	Coquet, C022989, 1973	Yes (still valid for AIR 3 according to EFSA/2009/1438)
	Rabbit	Rodwell, C032281, 1985	Yes (still valid for AIR 3 according to EFSA/2009/1438)

1 EU agreed means assessed during the previous EU evaluation process, but not necessarily listed in the list of endpoint as in the Review report for the active substance iprodione, 5036/VI/98-final, 03.12.02

CA 8.1.1 Effect on birds

CA 8.1.1.1 Acute oral toxicity to birds

No new study available (cp.Table 8.1-2).

CA 8.1.1.2 Short-term dietary toxicity to birds

No new study available (cp.Table 8.1-2).

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

No new study available (cp.Table 8.1-2).

CA 8.1.2 Effects on terrestrial vertebrates other than birds

CA 8.1.2.1 Acute oral toxicity to mammals

No new study available (cp.Table 8.1-2).

CA 8.1.2.2 Long-term and reproductive toxicity to mammals

One new two-generation rat study available (cp.Table 8.1-2). For summary of the study see respective toxicity chapter.

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 other 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 nor how to conduct a risk assessment for amphibian and reptiles. In the case of iprodione, no studies were found in the literature on the toxicity of this active ingredient on amphibians and reptiles.

According to the new aquatic guidance document (EFSA, 2013) amphibians 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 to 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). Regulatory ecotoxicological information on terrestrial amphibians is scarce in general. However, in the few cases where terrestrial stages of amphibians were tested in the same study as birds and mammals the general pattern is that amphibians are less sensitive than the latter two taxa (see Table 13 in Fryday and Thompson, 2012). This suggests that the quantitative risk assessment done for birds and mammals would be conservative for the terrestrial phase of amphibians.

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. This uncertainty will remain until appropriate and validated testing methods are developed and validated and adequate regulatory risk assessment schemes are implemented in the EU.

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

Mammals

Findings of potential relevance to endocrine disruption identified in repeated dose, investigative and mechanistic studies performed with iprodione are discussed in M-CA 5.8.3. Conclusions are drawn on a potential mode of action of iprodione and most sensitive endpoints identified. It is shown that the available dataset in its entirety is consistent, and identifies a threshold for endocrine related effects of iprodione (based on the analysis of a number of relevant endpoints). The overall NOEL is approximately 30 mg/kg b.w./d and the LOEL approximately 70 mg/kg b.w./d. For further details please see M-CA 5.8.3. For selection of the mammalian toxicity endpoint for use in the reproductive risk assessment for iprodione please see M-CP 10.1.2.

Birds

The question of endocrine and birds has been addressed by the notifier in a position paper [see 2014/1086097 Anonymous 2014 c], the conclusion of which is that the reproductive endpoint from the standard one-generation bird studies with iprodione is sufficiently protective to address the question of endocrine modulation for the risk assessment. Consequently, the toxicity value from the one-generation reproduction quail study with iprodione (NOEC = 300 mg/kg diet or 22.3 mg a.s./kg b.w./d) (BASF DocID R014619) is the appropriate toxicity endpoint for use in the avian reproductive risk assessment for iprodione.

The position paper on the selection of the appropriate bird toxicity endpoint for the bird reproductive risk assessment taking into account endocrine modulation [see 2014/1086097 Anonymous 2014 c] was not included in the Application of October 2013.

CA 8.2 Effects on aquatic organisms

Since Annex I inclusion of iprodione (BAS 610 F), new toxicity studies on the active substance and its major metabolites 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. Details on the EU agreed studies which have been already evaluated within the Annex I inclusion of iprodione are provided in the EU Review documents of iprodione (Monograph, Vol. 3, Annex B.8, June 1996; EU Review Report (5036/VI/98-final), December 2002).

For better transparency and traceability of the active substance history, the results of all studies are summarized in Table 8.2-1.

Full references used within the following chapters are given at the end of the document.

Table 8.2-1: Summary of the toxicity values of the active substance iprodione and its major metabolites for aquatic organisms

Organism	Endpoint	Value [mg/L] (except BMF/BCF)	Reference	EU agreed
active substance: iprodione (BAS 610 F)				
Fish				
Oncorhynchus mykiss	96 h LC_{50}	4.1	Sousa, C021726	yes
Lepomis macrochirus	96 h LC_{50}	3.7	Sousa, R014560	yes
Ictalurus punctatus	96 h LC_{50}	**3.1**	Swiegert, C021751	yes
Cyprinodon variegatus [2)]	96 h LC_{50}	7.7	C021728	yes
Pimephales promelas	34 d NOEC (ELS)	0.260	Surprenant, C021734	yes
P. promelas [1)]	56 d NOEC (partial LC)	**0.090**	[redacted] 2013/1311402	no (new study)
P. promelas (short-term reproduction assay) [1)]	23 d NOEC	0.0085	[redacted], 2012/1364403	no (US EPA EDSP Tier 1 data for US registration [#])
Xenopus laevis [1)]	21 d NOEC (AMA)	≥ 0.700	[redacted], 2012/1364404	no (US EPA EDSP Tier 1 data for US registration [#])

Organism	Endpoint	Value [mg/L] (except BMF/BCF)	Reference	EU agreed
Aquatic invertebrates				
Daphnia magna	48 h EC_{50}	**0.660** [3)]	McElligott, C021746	yes
D. magna	48 h EC_{50}	0.250 [3)]	McNamara R014562	yes
Americamysis bahia [1), 2), 4)]	48 h LC_{50}	> 0.970 +	Surprenant, C022006	no (old data for US registration; included for completeness)
Crassostrea virginica [1), 2)]	96 h EC_{50}	2.3	Surprenant, C021944	no (old data for US registration; included for completeness)
Procambarus simulans [1)]	7 d LC_{50}	> 4.1	McAllister & Bunch, C021999	no (old data for US registration; included for completeness)
D. magna	21 d NOEC	**0.170**	Surprenant, C021735	yes
Ceriodaphnia dubia	8 d NOEC	0.122	Hicks, 2013/7002669	no (new study)
Hyalella azteca	-- *	-- *	Hicks, 2013/7002670	no (new study)
A. bahia [1), 2), 4)]	28 d NOEC	< 0.015 (extrapolated: 0.0030)	Surprenant, C022008	no (old data for US registration; included for completeness)
A. bahia [1), 2), 4)]	28 d NOEC	**0.0075** [5)]	Surprenant, C026001	no (old data for US registration; included for completeness)

Organism	Endpoint	Value [mg/L] (except BMF/BCF)	Reference	EU agreed
Algae [6]				
Selenastrum capricornutum (Syn. *Pseudokirchneriella subcapitata*)	72 h E_rC_{50} / E_bC_{50}	**> 1.5** [7]	Giddings, R014561 + Amendment: Pallen, R014645	yes
Navicula pelliculosa [1]	120 h E_bC_{50}	0.048 [8]	Giddings, C021737	no (old data for US registration; included for completeness)
Anabaena flos-aquae [1]	120 h E_bC_{50}	> 1.3 [8]	Giddings, C021725	no (old data for US registration; included for completeness)
Skeletonema costatum [1), 2)]	120 h E_bC_{50}	0.590 [8]	Giddings, C021736	no (old data for US registration; included for completeness)
Aquatic macrophytes [6]				
Lemna gibba	14 d E_bC_{50}	> 1.5 [9]	Giddings, C022591	yes
L. gibba [1]	7 d E_rC_{50} / E_bC_{50}	**> 12.64** [9]	Sowig, C022577	no (included for completeness)
Bioconcentration / Metabolism				
Procambarus simulans (bioconcentration)	for results please refer to chapter 8.2.2.3 below		McAllister et al., C021930	no (old data, included for completeness)
P. simulans (metabolism & metabolite identification)	for results please refer to chapter 8.2.2.3 below		Wargo et al., C021931	no (old data, included for completeness)

Organism	Endpoint	Value [mg/L] (except BMF/BCF)	Reference	EU agreed
metabolite: RP 30228 (Reg. No. 5079647, M610F001)				
Fish				
O. mykiss	88 h LC_{50}	> 0.470	Bogers, C021762	yes
L. macrochirus	96 h LC_{50}	**0.550** [10)]	Bettencourt, C021739	yes
Aquatic invertebrates				
D. magna	48 h EC_{50}	> 0.500	Bogers, C021764	yes
Sediment dwellers				
Chironomus riparius (spiked water study)	28 d NOEC	≥ 0.140 [11)]	Odin-Feurtet, C022004	yes
C. riparius [1)] (spiked sediment study)	28 d NOEC	≥ 95.3 [12)]	Kuhl & Wydra, 2013/1000181	no (new study)
Algae [6)]				
Scenedesmus subspicatus	72 h E_rC_{50} / E_bC_{50}	> 0.500	Bogers, C021993	yes
metabolite: RP 36221 (Reg. No. 5079618, M610F002)				
Aquatic invertebrates				
D. magna [1)]	48 h EC_{50}	0.364	Swierkot, 2013/1191247	no (new study)
Algae [6)]				
P. subcapitata [1)]	72 h E_rC_{50} 72 h E_yC_{50}	**0.567** 0.154	Swierkot, 2013/1191246	no (new study)
metabolite: RP 25040 (Reg. No. 207099, M610F004)				
Aquatic invertebrates				
D. magna [1)]	48 h EC_{50}	70.54	Swierkot, 2013/1000223	no (new study)
Algae [6)]				
P. subcapitata [1)]	72 h E_rC_{50} 72 h E_yC_{50}	**97.64** 18.41	Swierkot, 2013/1000222	no (new study)
metabolite: RP 32596 (Reg. No. 85831, M610F012, 3,5-dichloroaniline (3,5- DCA))				
Fish				
Danio rerio	28 d LC_{50}	**1.3**	Van Leeuwen et al. (1990), 1990/1004212 [13)]	No (peer-reviewed scientific study)
	7 d NOEC	5.6		
	14 d NOEC	5.6		
	21 d NOEC	1.0		
	28 d NOEC	1.0		

Organism	Endpoint	Value [mg/L] (except BMF/BCF)	Reference	EU agreed
Aquatic invertebrates				
D. magna [1)]	48 h EC_{50}	1.26	Rzodeczko, 2013/1000225	no (new study)
Algae [6)]				
P. subcapitata [1)]	72 h E_rC_{50} 72 h E_yC_{50}	**9.25** 2.40	Rzodeczko, 2013/1000224	no (new study)

Bold figures: Where several endpoints are available for the same group or where several endpoints are available for one study based on different effect parameters (*e.g.* for algae and macrophytes), the relevant endpoint for the most sensitive species is used in the following TER calculations.

ELS = early life stage; LC = life cycle study; AMA = Amphibian Metamorphosis Assay;

[#] Screening studies; endpoints are not relevant for the following TER calculations. However, the results are reported here as additional information.

[+] The 48-h LC_{50} obtained in the 96 h study is used in the risk assessment according to EU Regulation 283/2013 (European Commission, 2013) on the data requirements for active substances and the EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA, 2013).

***** Study could not be finalized; for reasons see summary below.

1) Study has not been submitted during after Annex I inclusion process of iprodione; a study summary is provided below.

2) marine species

3) In accordance with the EU Review Report (5036/VI/98-final; December 2002) the higher endpoint from the study on *D. magna* performed by McElligott (BASF DocID C021746) was considered as relevant endpoint; the lower endpoint obtained in the study by McNamara (BASF DocID R014562) was not used due to the following reasons: the measured concentrations at test initiation in this study were only between 18% and 33% of nominal concentrations; the initially measured concentrations (day 0) were thus not used to establish mean measured test concentrations. Instead, mean measured concentrations were calculated using the actual analytical results from 24-hour (42% - 57% of nominal) and 48-hour (35% - 41% of nominal) analyses. Due to the difficulties in maintaining the correct concentrations during the study and as the calculations of mean measured concentration based on day 1 and 2 measured values seems questionable, the study is considered to be not valid

4) former name: *Mysidopsis bahia*

5) The second study on *A. bahia* (Surprenant, DocID C026001) replaces the former study (Surprenant, DocID C022008).

6) In accordance with recent guidelines for aquatic primary producers (*e.g.* EFSA Scientific Opinion (EFSA 2013); Aquatic Guidance Document (SANCO, 2002); OECD guideline 201 (OECD, 2011) and 221 (OECD, 2006)), only the EC_{50} values determined for the endpoint 'growth rate' (E_rC_{50}) are considered for the risk assessment for algae and macrophytes if both “growth rate” and “biomass” endpoints are available.

7) In accordance with current OECD guideline 201 (OECD, 2011) the 72-h growth rate endpoint from the 120-h alga study has been (re-)calculated from original data and is used in the following risk assessment; for details please refer to the respective amendment.

8) The studies on these alga species are considered to be not valid due to the reasoning given at the beginning of chapter CA 8.2.6.2 below. Nevertheless, summaries of these studies are provided for the sake of completeness.

9) The results of the older (EU agreed) study on *Lemna gibba* (Giddings, C022591) are not considered relevant for the aquatic risk assessment anymore since the test duration in this study deviates from the recommendations of current guidelines (*i.e.* test duration of 14 days instead of 7 days as recommended by EFSA (2013) and OECD (2006)); furthermore, only one test concentration was applied and no definitive endpoint could be determined at the tested concentration range (EC_{50} > highest tested concentration). Instead, the results of the more recent study performed on *Lemna gibba* (Sowig, DocID C022577; performed after Annex I inclusion of iprodione) according to current guidelines are considered in the aquatic risk assessment.

10) No clear endpoint could be determined in the acute study with *O. mykiss* (*i.e.,* mortality < 50% at the highest tested concentration); thus, the (greater) endpoint (LC_{50} = 0.550 mg/L) of the acute study with *L. macrochirus* is considered as relevant endpoint for the acute risk assessment for fish.

11) The NOEC value based on mean measured concentrations is considered to be more appropriate to be used in the risk assessment as the initially measured concentrations in the study were significantly higher than the nominal values (*i.e.* 119 - 165% of nominal).

12) mg/kg dry sediment

13) For the metabolite RP 32596 (3,5-dichloroaniline) a weight of evidence approach was applied by considering the relevant and reliable fish data available from literature.

CA 8.2.1 Acute toxicity to fish

No study required; thus, this point is not addressed *via* (new) toxicity studies.

CA 8.2.2 Long-term and chronic toxicity to fish

CA 8.2.2.1 Fish early life stage toxicity test

The following scientific peer-reviewed open literature study was not found via the global literature search process performed for the Annex I renewal process as studies older than 10 years were excluded due to the criteria defined in the search profiles. Instead, the study by Van Leeuwen et al. (1990) was subsequently included in the hit list and evaluated in accordance with the methods described in the Literature Search Report (see chapter CA 9). The early life stage study on *Danio rerio* was considered relevant and reliable (with restrictions; RI 2) for the aquatic risk assessment of the iprodione metabolite RP 32596 (for details please refer to Document K of this dossier). Thus, it is provided in support of the aquatic risk assessment and has not been evaluated previously.

Report:	CA 8.2.2.1/1 Leeuwen C.J. van et al., 1989a Quantitative structure-activity relationships for fish early life stage toxicity 1990/1004212
Guidelines:	none
GLP:	no

Executive Summary

Early life stage (ELS) toxicity experiments were carried out for the aniline derivative 3.5-dichloroaniline under semi-static conditions using zebra fish (*Danio rerio*). The parameters studied included survival, embryo-hatchability and growth.

Retardation of growth appeared to be the most sensitive parameter. Although a number of embryo toxic effects such as skeletal deformities, enlarged yolk sacs and edemas, were frequently observed in the higher test concentrations. These data were not treated statistically as most of the larvae involved had died before the end of the test period. Moreover, these developmental effects occurred at concentrations higher than those which significantly affected growth.
The concentrations of 3.5-dichloroaniline remained constant during the tests and deviated less than 10% from the nominal concentrations. Therefore, all results and calculations were based on nominal concentrations.

In an early life stage study with zebra fish (*Danio rerio*), the 28 day NOEC for 3.5-dichloroaniline was determined to be 1.0 mg/L based on nominal concentrations. The 28 day LC_{50} was 1.3 mg/L, based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: 3.5-dichloroaniline (supplier: Fluka AG), purity > 97%, log P_{oct} 2.88 (data taken from De Bruijin et al., 1989): Some relevant information (batch no.; density, etc.) is missing in the study report.

B. STUDY DESIGN

Test species: Zebra fish (*Danio rerio,* formerly known as *Brachydanio rerio*), fertilized eggs in the blastula stage were obtained from a stock culture at the TNO laboratory

Test design: Semi-static system (28 d); 7-8 test item concentrations plus a solvent (dimethyl sulphoxide, DMSO) control and a control, 2 replicates per concentration, 50 -100 fertilized eggs (< 6 h after spawning) were transferred into l L glass test vessels filled with 1 L test solution. After 1 day all non-viable eggs were removed and the number of viable eggs was reduced to a maximum of 40 per concentration. Upon completion of hatching (4-5 days), the fry were transferred into two vessels per concentration. The test solutions were renewed 3 times a week. Dead eggs and larvae were counted and removed daily. At the end of the test period the surviving fish were anesthetized in buffered tricaine methane sulphonate (MS 222, Sandoz, Basel) for final length measurements. The number of microscopically malformed fish was determined under a microscope.

Endpoints: EC_{50} values and NOEC values based on survival, growth and embryo-hatchability.

Test concentrations: Water control, solvent (dimethyl sulphoxide, DMSO) control, 7 - 8 toxicant concentrations. The ratio between the concentrations was 1.8.

Test conditions: Test vessels: glass vessels, water volume: 1 L; dilution water: groundwater obtained from a locality near Linschoten, to which several salts were added, temperature: 24 ± 2 °C; pH values: 7.4 - 8.4; oxygen content: 5.1 mg/L - 7.7 mg/L; total hardness: 210 mg $CaCO_3$/L; photoperiod: 12 hours light : 12 hours dark. Feeding: fry were fed equal amounts of the rotifer *Brachionus rubens,* obtained from a laboratory culture; after 7 days this food was supplemented by 48-h old nauplii of the brine shrimp *Artemia salina; t*he nauplii were enriched with Selco, a commercial concentrate for nutritional enrichment of live food for fish; slight aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics. The LC_{50} and 95% confidence limits were calculated according to Kooyman (1981). For calculation of NOEC values a two-stage approach was applied to exclude any possible effects of size-selective mortality.

II. RESULTS AND DISCUSSION

Analytical measurements: The actual concentrations of the test compounds were verified before and after renewal of the test solutions during the experiments. The concentrations of 3.5-dichloroaniline remained constant during the tests and deviated < 10% from the nominal concentrations. Therefore, all results and calculations were based on nominal concentrations.

Biological results: Retardation of growth appeared to be the most sensitive parameter. Although a number of embryo toxic effects such as skeletal deformities, enlarged yolk sacs and edemas, were frequently observed in the higher test concentrations, these data were not treated statistically as most of the larvae involved had died before the end of the test period. Moreover, these developmental effects occurred at concentrations higher than those which significantly affected growth. The results are summarized in Table 8.2.2-1.

Table 8.2.2-1: Results of the early life stage test with *D. rerio* and 3.5-dichloroaniline

Endpoint	Value in mg/L
28 d LC_{50}	1.3 (95% confidence limits: 1.0 - 1.8)
7 d NOEC	5.6
14 d NOEC	5.6
21 d NOEC	1.0
28 d NOEC	1.0

III. CONCLUSION

In an early life stage study with zebra fish (*Danio rerio*), the 28 day NOEC for 3.5-dichloroaniline was determined to be 1.0 mg/L based on nominal concentrations. The 28 day LC_{50} was 1.3 mg/L, based on nominal concentrations.

CA 8.2.2.2 Fish full life cycle test

No study required; thus, this point is not addressed *via* (new) toxicity studies.

CA 8.2.2.3 Bioconcentration in fish

The following bioconcentration studies performed with iprodione are provided for the sake of completeness and have not been evaluated previously.

Report:	CA 8.2.2.3/1 McAllister W.A. et al., 1986a Bioconcentration and depuration of 14C-Iprodione by crayfish (Procambarus simulans, Faxon) under static uptake conditions with a treated soil substrate C021930
Guidelines:	EPA
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The bioconcentration potential of ^{14}C-iprodione (BAS 610 F) was investigated in crayfish (*Procambarus simulans*). The test item was incorporated into a sandy loam at a single nominal concentration of 2.0 mg a.s./kg dried soil. Animals were exposed over a 28-day uptake phase under static condition. Subsequently, crayfish were placed in dilution water only in a flow-through system for 14 days (depuration phase). A control population of crayfish was tested under the same test conditions, however without test item application. The concentrations in crayfish and water were determined on several occasions during the uptake phase and the depuration phase. Concentrations in soil were measured in samples collected during aging, equilibration and uptake.

The measured ^{14}C soil residues decreased from 1.9 mg/kg at day 28 of the aging period to 0.94 mg/kg at day 28 of the uptake phase (51% reduction). During the 3-day soil/water equilibration period the ^{14}C-iprodione levels decreased from 1.9 mg/kg to 1.4 mg/kg (26% reduction). No reduction in ^{14}C-iprodione residues was observed during the 28-day soil aging period, where residues averaged 1.7 to 1.9 mg/kg. The mean water concentrations ranged from a low of 0.0076 mg/L on day 0 to a high of 0.026 mg/L on day 14 of the uptake phase. Water residue levels remained basically unchanged from day 7 to day 28 of the uptake period, ranging from 0.023 to 0.027 mg/L. Al1 water levels during the depuration period were below minimum quantifiable limits. Uptake phase tissue concentrations ranged from 0.094 to 0.25 mg/kg in edible tissue and 0.22 to 0.48 mg/kg in whole crayfish. The bioconcentration factors ranged from 6.2x to l0x for edible tissue and 8.8x to 20x for whole crayfish. Based upon edible tissue levels, a steady-state plateau was reached by day 10 of the uptake phase. To measure the elimination of the test item, the crayfish were placed in clean flowing water for 14 days. Radioanalysis throughout the depuration period indicated 84% and 59% ^{14}C-residue elimination by day 14 depuration from edible and whole crayfish tissue, respectively. No toxic effects (*i.e.* mortality) or changes in behavior were observed in the test treatment organisms in comparison to the control group.

In a static bioconcentration study, crayfish were exposed to radiolabeled iprodione at a concentration of 2.0 mg a.s./kg (nominal) in sandy loam soil. Uptake phase tissue concentrations of ^{14}C-iprodione ranged from 0.094 to 0.25 mg/kg in edible tissue and 0.22 to 0.48 mg/kg in whole crayfish. The bioconcentration factors ranged from 6.7x to 10x for edible tissue and 8.8x to 20x for whole crayfish, when based on the respective daily measured water concentration. Based upon edible tissue levels a steady-state plateau was reached by day 10 of the uptake phase.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Radiolabelled iprodione (^{14}C-BAS 610 F, Reg. No. 101 169); phenyl ring labelled), lot no.: RP2024-111-1, specific activity 2.326 mCi/mmol), radiochemical purity: 97%.

B. STUDY DESIGN

Test species: Crayfish (*Procambarus simulans*, Faxon), initial mean body weight: 5.8 g ± 2.0 g; initial mean total length: 60 mm ± 5.8 mm, source: Northrup Hatchery in Centralia, Missouri, USA.

Test design: 28 d uptake phase under static conditions followed by a 14 d depuration phase under flow-through conditions; one test item concentration plus an untreated control; one tank per treatment with 130 crayfish per tank at the start of the uptake period.
Soil-Aging and Equilibration Period: Both the treated and control tanks filled with soil were aged aerobically for 28 days. During this time, the soil moisture was maintained at approximately 75% field capacity, or greater, by daily addition of ABC well water as a fine spray. At the end of the 28-day soil aging period, the tanks were filled with 300 L test water and allowed to equilibrate for 3 days.
Crayfish Exposure and Depuration Phases: After the 3-day equilibration period, crayfish were added to both the control and treated tanks. At the termination of the 28-day exposure period, the remaining crayfish in each tank were transferred to the depuration tanks containing clean test water for additional 14 days of depuration.

	Sampling: Water and crayfish were sampled for radioactivity measurement on several occasions during the uptake phase and depuration phase. At each sampling time, seven crayfish were removed from each test tank or glass aquaria. After sampling, crayfish were sacrificed and 4 crayfish were dissected into edible tissue and viscera. Three crayfish from each tank were reserved for whole crayfish analysis. Additional crayfish were collected for metabolite characterization on certain sampling days. Soil samples were collected on four days of aging, on one day of equilibration and on eight days of uptake. All samples were kept frozen until radioanalysis. Additionally, crayfish behaviour and survival was assessed.
Endpoints:	Bioconcentration potential (bioconcentration factor, BCF), elimination.
Test concentrations:	Control (untreated soil), 2.0 mg iprodione/kg dried soil (nominal).
Test conditions:	Bioconcentration tanks for aging and uptake phase (148 cm x 57 cm x 60 cm) containing mesh wire cages during the uptake phase(61 cm x 46 cm x 30 cm): test water: 300 L aerated ABC well water;test soil: non-sterilized sandy loam soil (73%, silt 23%, clay 4%), average depth 2.0 cm; giving a ratio of 1 L soil:42 L test water; continuous aeration; 16 hours light : 8 hours dark; temperature: 18 °C - 20 °C; oxygen content: 8.4 mg/L - 9.1 mg/L; pH 8.1 - 8.4. Depuration tanks (100 L glass aquaria) (containing 75 L aerated ABC well water; flow-rate:300 ml/minute/aquarium (sufficient to replace 75 L approx. 5 times in a 24 h period).; temperature: 19 °C; pH 8.0 - 8.2; oxygen content 7.5 mg/L - 9.1 mg/L; 16 hours light : 8 hours dark; Feeding: commercial fish diet (Rangen´s) *ad libitum* during test period.
Analytics:	Determination of test item concentrations in water, soil and crayfish tissue samples was conducted by radioactivity measurement using Liquid Scintillation Counting (LSC).

II. RESULTS AND DISCUSSION

Test item concentration in the water
The water concentrations ranged from 0.0076 mg ^{14}C-iprodione/L on day 0 to a high of 0.026 mg ^{14}C-iprodione/L on day 14 of the uptake phase. Water residue levels remained basically unchanged from day 7 to day 28 of the uptake period (0.023 - 0.026 mg ^{14}C-iprodione /L). All water levels during the depuration phase were below minimum quantifiable limits. The initial increase in water concentration with respect to time during the uptake period can be attributed to the desorption of the ^{14}C-iprodione from the soil.

Test item concentration in soil
During day 28 of the aging period to day 28 of the uptake period the concentration of the ^{14}C-residues decreased from 1.9 mg/kg to 0.94 mg/kg. This represented a 51% reduction in ^{14}C-radioactivity in soil. A 26% reduction was observed during the equilibration period (1.9 mg/kg to 1.4 mg/kg). No reduction in ^{14}C-residues was observed during the soil aging period (average 1.7 to 1.9 mg/kg).

Test item concentrations in crayfish
Uptake phase tissue concentrations of ^{14}C-iprodione ranged from 0.094 to 0.25 mg/kg in edible tissue and 0.22 to 0.48 mg/kg in whole crayfish. The bioconcentration factors ranged from 6.7x to 10x for edible tissue and 8.8x to 20x for whole crayfish, when based on the respective daily measured water concentration. Based upon edible tissue levels a steady-state plateau was reached by day 10 of the uptake phase. An analysis of clearance rates by day 14 of the depuration period showed 84% and 59% depuration in edible and whole crayfish, respectively. The depuration data indicated that residues were basically retained for the first 3 days of the depuration period, followed by increased elimination for the remainder of the study. After day 3, the data indicated a gradual clearance of ^{14}C as iprodione from crayfish edible tissues up to test termination at day 14 of depuration. The non-edible tissue had ^{14}C-iprodione concentrations of 0.61 mg/kg and 0.12 mg/kg on days 28 of the uptake phase and day 14 of the depuration phase, respectively.

General observations
No toxic effects like increased mortality or changes in behavior were observed in the test item treatment organisms in comparison to the control group.

The bioconcentration factors (BCF) and depuration values for the whole crayfish and for edible tissues based on measured data are summarized in Table 8.2.2.3-1 and Table 8.2.2.3-2.

Table 8.2.2.3-1: Bioconcentration factors (BCF) of ^{14}C-iprodione in crayfish tissue in the presence of treated soil substrate

Study day		Water [mg/L]	Soil [mg/kg]	Edible tissue (BCF) *	Whole crayfish (BCF)*	Time weighted mean water [mg/L]	Edible tissue (BCF) #	Whole crayfish (BCF) #
Aging	0	--	1.7	--	--	--	--	--
	1	--	1.7	--	--	--	--	--
	15	--	1.9	--	--	--	--	--
	28	--	1.9	--	--	--	--	--
Equilibration	1	--	1.4	--	--	--	--	--
Uptake	0	0.0076	1.4	--	--	--	--	--
	1	0.014	1.3	6.7x	20x	0.011	8.5x	25x
	3	0.016	1.2	6.2x	20x	0.013	7.7x	25x
	7	0.023	1.0	7.0x	20x	0.015	11x	31x
	10	0.025	1.0	8.0x	15x	0.017	12x	22x
	14	0.026	0.84	7.3x	18x	0.019	10x	25x
	21	0.025	1.0	7.6x	8.8x	0.020	9.5x	11x
	28	0.025	0.94	10x	12x	0.020	12x	14x

* BCF obtained using the respective sample day measured water concentration.
# BCF obtained using the mean measured water concentration up to that respective sample day.

Table 8.2.2.3-2: Depuration* of ^{14}C-iprodione from crayfish during a 14-day clearance period

Depuration day	Edible tissue		Whole crayfish	
	Depuration concentration [ppm]	Percent depuration	Depuration concentration [ppm]	Percent depuration
1	0.14	44	0.34	-- #
3	0.21	16	0.44	-- #
7	0.079	68	0.12	59
10	0.051	80	0.33	-- #
14	0.039	84	0.12	59

* percentage of the day 28 14C-iprodione concentration of 0.25 ppm for edible tissue and 0.29 ppm for whole crayfish
# no calculation possible since residue levels were higher than the day 28 uptake values

III. CONCLUSION

In a static bioconcentration study, crayfish were exposed to radiolabeled iprodione at a concentration of 2.0 mg a.s./kg (nominal) in sandy loam soil. Uptake phase tissue concentrations of ^{14}C-iprodione ranged from 0.094 to 0.25 mg/kg in edible tissue and 0.22 to 0.48 mg/kg in whole crayfish. The bioconcentration factors ranged from 6.7x to 10x for edible tissue and 8.8x to 20x for whole crayfish, when based on the respective daily measured water concentration. Based upon edible tissue levels a steady-state plateau was reached by day 10 of the uptake phase.

The following study is part of the bioconcentration study reported above (McAllister et al., 1986; BASF DocID C021930). The biological portion of the study, which included the treatment and total residue analysis to determine the bioconcentration potential, is described above, whereas the analytical portion, the determination of total extractable residues and metabolite identification, is reported within the following summary. The majority of the information on the test design and the materials used provided within the summary above is also valid for the following study and these parts are thus, not repeated in the summary below.

Report: CA 8.2.2.3/2
Wargo J.P. et al., 1986a
Identification of Iprodione residues in crayfish
C021931
Guidelines: none
GLP: no

Executive Summary

The following study was designed to determine the bioconcentration effects in crayfish (*Procambarus simulans*) exposed to water and soil treated with ^{14}C-iprodione. A secondary objective was to determine the metabolic pathway for iprodione in crayfish. The biological portion of the study, which included the treatment and total residue analysis to determine the bioconcentration, was completed by another laboratory and is described within the summary above. The analytical portion, the determination of total extractable residues and metabolite identification, is described here. A detailed description of the test design and the materials used in this study is provided in the study summary above. Briefly, the test item was incorporated into a sandy loam at a single nominal concentration of 2.0 mg a.s./kg dried soil. Animals were exposed over a 28-day uptake phase under static condition. Subsequently, crayfish were placed in dilution water only in a flow-through system for 14 days (depuration phase). A control population of crayfish was tested under the same test conditions, however without test item application. The concentrations in crayfish and water were determined on several occasions during the aging, uptake and the depuration phase. Metabolite characterization in water, soil and crayfish was performed by thin-layer chromatography (TLC).

The total residue values for the soil ranged from 0.69 to 1.45 mg/kg, while the water values remained consistent (0.026 - 0.027 mg/kg) between 14 and 28 days. The crayfish edible tissue contained total residues of 0.23 to 0.25 mg/kg at 21 and 28 days while the non-edible tissue residues were 0.58 to 0.70 mg/kg for the same sampling periods. After a 14-day depuration, the total residues fell to 0.04 mg/kg for the edible tissue and to 0.09 mg/kg for the non-edible tissue. The extractability of the residues from the edible tissue ranged from 97 to 99%. The non-edible tissue residue extractability ranged from 67 to 91%. Over 75% of the residue in the water was extracted while the soil extractables ranged from 65 to 83% of the total ^{14}C-residue.
The major metabolite identified in water and soil was the iprodione isomer RP 30228 which comprised 32 to 51% of the total residue. Significant amounts of RP 26019 (10 - 17%) were also present in both the water and soil. RP 26019, RP 30228 and RP 44247 (3,5-dichlorophenyl-urea) were found in edible crayfish tissue in quantities ranging from 9 - 17% of the total residue. Significant amounts of activity (32 - 45% of the total residues) remained at the origin or moved just beyond the origin. The non-edible tissues from the uptake phase contained the two mayor metabolites RP 26019 and RP 30228 at concentrations representing 16 - 24% and 10 - 16% of total residues, respectively. The major metabolite found in edible and non-edible tissues from the depuration phase was RP 44247 accounting for 54 and 33% of the total residue respectively. After 14 days of depuration in clean flowing water, only RP 44247 remains in any significant quantities in both the edible and non-edible tissues.

In a static bioconcentration study, crayfish were exposed to radiolabeled iprodione at a concentration of 2.0 mg a.s./kg (nominal) in sandy loam soil. The metabolite characterization shows that RP 44247 is the only major iprodione metabolite in crayfish. Since RP 30228 is the major compound found in the water and soil, it is readily available for uptake by the crayfish.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: For details on the test item please refer to the information provided in the study summary above.

B. STUDY DESIGN

Test species: For details on the test species please refer to the information provided in the study summary above.

Test design: For details on the test design and samplings please refer to the information provided in the study summary above.

Endpoints: Determination of total ^{14}C-residues; identification of iprodione residues and determination of metabolic pathways in crayfish.

Test concentrations: Control (untreated soil), 2.0 mg ^{14}C-iprodione/kg dried soil (nominal), corresponding to 1.6 mg iprodione/kg dried soil (measured).

Test conditions: For details on the test conditions please refer to the information provided in the study summary above.

Analytics: Determination of test item concentrations in water, soil and crayfish tissue samples was conducted by radioactivity measurement using Liquid Scintillation Counting (LSC); metabolite characterization by thin-layer chromatography (TLC) using final ethyl acetate fractions obtained by specific extractions.

II. RESULTS AND DISCUSSION

Total ^{14}C-residues in water, soil and crayfish

The total ^{14}C-residues determined during this part of study showed good correlation with the data obtained in the other part (biological portion) of this study (compare study summary above). The total residue values for the soil ranged from 0.69 to 1.45 mg/kg, while the water values remained consistent (0.026 - 0.027 mg/L) between 14 and 28 days. For the purpose of quantitating the TLC results, total residues (TR) were obtained by adding the extraction values (TER) and the bound residues remaining in the filter cake. The crayfish edible tissue contained total residues of 0.23 to 0.25 mg/kg at 21 and 28 days while the non-edible tissue residues were 0.58 to 0.70 mg/kg for the same sampling periods. After a 14-day depuration, the total residues fell to 0.04 mg/kg for the edible tissue and to 0.09 mg/kg for the non-edible tissue. The extractability of the residues from the edible tissue ranged from 97 to 99%. The non-edible tissue residue extractability ranged from 67 to 91%. Over 75% of the residues in the water was extracted while the soil extractables ranged from 65 to 83% of the total ^{14}C-residues.

Metabolites in water, soil and crayfish

The major metabolite identified in water and soil was the iprodione isomer RP 30228 which comprised 32 to 51% of the total residues. Significant amounts of RP 26019 (10 - 17%) were also present in both the water and soil. RP 26019, RP 30228 and RP 44247 (3,5-dichlorophenyl-urea) were found in edible crayfish tissue in quantities ranging from 9 - 17% of the total residues. Significant amounts of activity (32 - 45% of the total residues) remained at the origin or moved just beyond the origin. The non-edible tissues from the uptake phase contained the two mayor metabolites RP 26019 and RP 30228 at concentrations representing 16 - 24% and 10 - 16% of total residue, respectively. The major metabolite found in edible and non-edible tissues from the depuration phase was RP 44247 accounting for 54 and 33% of the total residue respectively. After 14 days of depuration in clean flowing water, only RP 44247 remains in any significant quantities in both the edible and non-edible tissues.

The total ^{14}C-residues found in water, soil and crayfish are summarized in Table 8.2.2.3-3 and the metabolite distribution is shown in Table 8.2.2.3-4.

Table 8.2.2.3-3: The total (TR) and total extractable ^{14}C-residues found in water, soil and crayfish

Time interval days	Water [mg/L]			Soil [mg/kg]			Crayfish			
							Edible [mg/kg]		Non-edible [mg/kg]	
	Total ^{14}C-residues	TR *	TER as % of TR	Total ^{14}C-residues	TR *	TER as % of TR	TR *	TER as % of TR	TR *	TER as % of TR
Aging										
28	--	--	--	1.45	1.640	84	--	--		--
Uptake										
14	0.027	0.025	80	--	--	--	--	--	--	--
21	0.027	0.024	79	0.78	0.865	65	0.230	99	0.580	91
28	0.026	0.025	76	0.69	0.773	72	0.251	99	0.701	84
Depuration										
14	< 0.01	< 0.01	--	--	--	--	0.038	97	0.094	67

* calculated by adding the extraction values (TER) and the bound residues remaining in the filter cake (TBR).

Table 8.2.2.3-4: Metabolite distribution in water, soil and crayfish

Sample type	Time interval days	metabolite distribution [% of TR]								% of TR total
		Origin	RP 26019	RP 30228	RP 32490	RP 44247	RP 36112	RP 36114	Other metab.	
Soil										
Aging	28	1.44	17.31	51.35	1.99	5.25	3.69	--	3.71	84.7
Uptake	21	1.11	10.05	46.28	0.94	1.22	3.35	--	2.01	65.0
	28	1.26	13.86	48.63	1.13	0.84	3.64	--	2.53	71.9
Water										
Uptake	14	1.90	13.32	32.40	--	10.02	9.27	4.16	7.80	78.9
	21	1.88	12.00	41.69	--	9.47	5.94	2.12	9.46	82.6
	28	1.93	10.73	43.36	--	8.29	5.30	2.26	6.25	78.1
Crayfish (edible)										
Uptake	21	30.88	8.69	9.09	3.33	13.07	7.97	6.29	14.62	93.9
	28	13.01	14.14	15.26	2.41	17.44	6.36	7.23	19.20	95.0
Depur-ation	14	11.67	5.53	1.84	9.20	54.03	--	9.83	4.91	97.1
Crayfish (non-edible)										
Uptake	21	7.68	23.57	10.21	2.08	6.29	8.83	7.63	6.08	72.4
	28	7.24	16.29	15.61	1.77	7.84	6.84	5.92	14.38	75.9
Depur-ation	14	7.81	2.99	3.84	4.22	33.22	5.50	1.76	4.10	63.4

III. CONCLUSION

In a static bioconcentration study, crayfish were exposed to radiolabeled iprodione at a concentration of 2.0 mg a.s./kg (nominal) in sandy loam soil. The metabolite characterization shows that RP 44247 is the only major iprodione metabolite in crayfish. Since RP 30228 is the major compound found in the water and soil, it is readily available for uptake by the crayfish.

CA 8.2.3 Endocrine disrupting properties

The following short-term reproduction assay performed with iprodione on *Pimephales promelas* was performed as US EPA EDSP Tier 1 screening study for US registration. It is not required for registration in the EU and thus, it has not been evaluated previously on EU level. Nevertheless, the summary of this study is included below for the sake of completeness.

Report:	CA 8.2.3/1 [redacted]., 2012a Iprodione - Short-term reproduction assay with fathead minnow (Pimephales promelas) following OPPTS 890.1350 and OECD 229 guidelines 2012/1364403
Guidelines:	EPA 890.1350, OECD 229
GLP:	yes (certified by United States Environmental Protection Agency) [see 2014/1049126 Shearer K.S. 2014 c]

Executive Summary

The potential endocrine activity of iprodione in fathead minnow (*Pimephales promelas*) was evaluated in a 23-day short-term reproduction assay under flow-through conditions. Fish were exposed to a dilution water control and to nominal test item concentrations of 0.0085, 0.085 and 0.85 mg iprodione/L (equivalent to time-weighted average concentrations of 0.0086, 0.084 and 0.60 mg iprodione/L). The evaluated endpoints were survival, fecundity, fertilization success, nuptial tubercle score, fish weight and length, blood plasma vitellogenin (VTG) concentration, gonadosomatic index (GSI), histological examination of gonadal tissues as well as behavior and appearance, including secondary sexual characteristics.

The biological results are based on time-weighted average concentrations of iprodione. Percent survival of males, females and the survival rates based on combined data sets in the controls were 100%, 85% and 92%, respectively. Survival rates for female and male fish as well as the combined survival rates were not statistically significantly affected compared to the control at any test item concentration tested. No statistically significant effects on fish weight and length, Gonadal Somatic Indices, blood plasma vitellogenin concentrations compared to the control were observed at any test concentration. The percentage of fertilized eggs in the control and the test item concentrations of 0.0086, 0.084 and 0.60 mg a.s./L was 92%, 89%, 92% and 81%, respectively. The percentage of fertilized eggs was statistically significantly decreased at the highest tested concentration of 0.60 mg a.s./L when compared to the control. The mean number of eggs per female per day was statistically significantly decreased in the two highest tested concentrations of 0.084 and 0.60 mg a.s./L when compared to the control. Tubercles were not observed in females and they were thus not scored. For males, no statistically significant differences in mean tubercle scores compared to the control were determined in any tested concentration. Furthermore, no notable abnormalities were observed with regard to behavior, coloration/banding, changes in ovipositor appearance or size of dorsal nape pad. The histopathological analyses showed increased incidence of interstitial cell hyperplasia in exposed males. However, these effects did not show a dose-dependency. In female fish, no clearly identified lesions associated with the iprodione exposure were detected.

In a short-term reproduction assay with fathead minnow (*Pimephales promelas*), the overall NOEC (23 d) for iprodione was determined to be 0.0085 mg a.s./L based on time-weighted average concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Iprodione (BAS 610 F, Reg. no. 101 169), batch no. SIN0000217 (AE F062470-01-ß8), purity: 98.1%.

B. STUDY DESIGN

Test species: Fathead minnow (*Pimephales promelas*), obtained from an in-house laboratory supply of reproductively mature animals, approx. 21 weeks old at test initiation, in spawning condition; mean male weight: 3.1 g (2.7 - 3.9 g); mean female weight: 1.5 g (1.2 - 1.6 g).

Test design: Flow-through system (23 d); 3 test item concentrations plus a dilution water control, 4 replicates per treatment with four female and two male fish in each. The exposure system consisted of a 2 L intermittent flow proportional diluter and two tiered water baths. Prior to adding fish to the exposure system, subsamples of both males and females in the test population were weighed. During the exposure period, survival, toxic signs (appearance and behavior), fecundity and fertilization success were assessed daily. At test termination, fish were sacrificed, measured for total length and wet weight and observed for secondary sexual characteristics. Blood samples were taken for plasma vitellogenin (VTG) analysis and the gonads were fixed in-situ and then removed and weighted for gonadosomatic index (GSI) determination and histological analyses. Fish were preserved for subsequent tubercle scoring.

Endpoints: NOEC values based on survival (male, female and combined), toxic signs (behavior and appearance, including secondary sex characteristics), fecundity (number of eggs/female/day), fertilization success, nuptial tubercle score, vitellogenin (VTG) concentration (male and female), gonadosomatic Index (GSI, male and female), histological examination of gonadal tissues and weight and length (male and female).

Test concentrations: Control (dilution water), 0.0085, 0.085 and 0.85 mg iprodione/L (nominal); equivalent to time-weighted average concentrations of 0, 0.0086, 0.084 and 0.60 mg iprodione/L.

Test conditions:	20 L glass aquaria (39 x 20 x 25 cm) positioned in water baths; test volume: 10 L; dilution water: aerated well water; flow rate: 0.5 L/cycle/aquarium at a rate of approx. 132 cycles/24 h (providing a turnover rate of 6.6 volume replacements every 24 h); water temperature: 24 °C - 26 °C; pH: 6.7 - 7.5; oxygen saturation: 61% - 96%; total hardness: 52 - 56 mg $CaCO_3$/L; conductivity: 280 - 310 µS/cm; alkalinity: 22 - 26 mg $CaCO_3$/L; light intensity: approx. 540 - 1100 lux; photoperiod: 16 h light : 8 h dark; feeding: from day 0 to day13 fish were fed twice a day with frozen brine shrimp nauplii (*Artemia*), from day 14 onwards live brine shrimp nauplii were fed twice a day.
Analytics:	Analytical verification of iprodione concentrations was conducted using an HPLC-method with UV detection.
Statistics:	Descriptive statistics; Fisher Exact/Bonferroni-Holm Test for survival data; Dunnett's Multiple Comparison Test for fertilization success, GSI, VTG and weight data; Jonckheere-Terpstra Step-Down Test for fecundity and tubercle score data.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical measurements of the test substance concentrations in the application solutions of each treatment were conducted at test initiation, on days, 9, 16, 18 and at test termination. The mean measured concentrations of iprodione measured in the exposure solutions taken at test initiation ranged from 92.9% to 111.2% of the nominal concentrations. Mean measured water concentrations of iprodione in all test item treatments were between 84.7% and 104.7% of nominal 9 days after application and between 61.8% and 110.6% of nominal on day 16. After 18 days, mean measured concentrations were between 44.1% and 92.4 of nominal for all treatment levels. At exposure termination concentrations ranged from 40.0% to 103.5% of nominal. The mean recovery rates over the whole study period were 100%, 98% and 71% of nominal in the test concentrations of 0.0085, 0.085 and 0.85, respectively. The lowest recovery was found in the highest test item concentration at each sampling, indicating rapid dissipation of iprodione from the water column, probably by adsorption due to increasing biomass in the aging system and spawning activity. Since the sampling times were not equally spaced and exposure concentrations decreased slightly over the course of the exposure in the higher test concentrations, a time-weighted average (twa) was calculated for each treatment level and the following biological results are based on the calculated twa concentrations.

Biological results: Percent survival of males, females and the survival rates based on combined data sets in the controls were 100%, 85% and 92%, respectively. Survival rates for female and male fish as well as the combined survival rates were not statistically significantly affected compared to the control at any test item concentration tested (Fisher Exact/Bonferroni-Holm Test). No statistically significant effects on fish weight and length, Gonadal Somatic Indices, blood plasma vitellogenin concentrations compared to the control were observed at any test concentration (Dunnett's Multiple Comparison Test). The percentage of fertilized eggs in the control and the test item (time-weighted average) concentrations of 0.0086, 0.084 and 0.60 mg a.s./L was 92%, 89%, 92% and 81%, respectively. The percentage of fertilized eggs was statistically significantly decreased at the highest tested concentration of 0.60 mg a.s./L when compared to the control (Dunnett's Multiple comparison Test). The mean number of eggs per female per day was statistically significantly decreased in the two highest tested concentrations of 0.084 and 0.60 mg a.s./L when compared to the control (Jonckheere-Terpstra Step-Down Test). Tubercles were not observed in females; therefore, they were not scored. For males, no statistically significant differences in mean tubercle scores compared to the control were determined in any tested concentration. Furthermore, no notable abnormalities were observed with regards to behavior, coloration/banding, changes in ovipositor appearance or size of dorsal nape pad. The histopathological analyses showed increased incidence of interstitial cell hyperplasia in exposed males. However, these effects did not show a dose-dependency. There were no clearly identified lesions associated with the iprodione exposure in female fish. The results are summarized in Table 8.2.3-1.

Table 8.2.3-1: Chronic toxicity and endocrine activity of iprodione to fathead minnow (*Pimephales promelas*) in a short-term reproduction assay (23 d)

Concentration [mg a.s./L] (nominal)	**Control**	**0.0085**	**0.085**	**0.85**
Concentration [mg a.s./L] (time-weighted average)	**Control**	**0.0086**	**0.084**	**0.60**
Male survival [%]	100	100	88	72
Female survival [%]	85	81	94	72
Combined (male& female) survival [%]	92	88	92	72
Fertilization success [% fertilized eggs]	92	89	92	81 *
Fecundity [number of eggs/female/day]	20	16	11 #	9.4 #
Mean male length on day 23 [mm]	63	65	63	64
Mean female length on day 23 [mm]	51	52	52	52
Mean male weight on day 23 [g]	3.02	3.30	2.83	2.69
Mean female weight on day 23 [g]	1.42	1.49	1.53	1.51
Mean male GSI [%]	1.3	1.1	1.8	1.4
Mean female GSI [%]	13	12	13	12
Male VTG concentration [ng/mL]	85	53	9252	15204
Female VTG concentration [ng/mL]	0.902×10^6	1.19×10^6	0.791×10^6	0.985×10^6
Male median tubercle score	34	28	37	41
Symptoms +	none	none	none	none
	Endpoints [mg a.s./L] (time-weighted average)			
$NOEC_{overall}$ (23 d)	0.0086			

GSI = Gonadal Somatic Index (gonad weight/body weight x 100)
* Statistically significantly different compared to the control (Dunnett's Multiple Comparison Test)
\# Statistically significantly different compared to the control (Jonckheere-Terpstra Step-Down Test)
\+ Symptoms: fish were observed for behavior, coloration/banding, changes in ovipositor appearance or size of dorsal nape pad.

III. CONCLUSION

In a short-term reproduction assay with fathead minnow (*Pimephales promelas*), the overall NOEC (23 d) for iprodione was determined to be 0.0085 mg a.s./L based on time-weighted average concentrations.

The following fish partial life-cycle test performed with iprodione on *Pimephales promelas* is provided in support of the aquatic risk assessment and has not been evaluated previously. The study was performed for assessing effects on iprodione on fish reproduction.

Report:	CA 8.2.3/2 [redacted]., 2014a BAS 610 F (Iprodione) - Partial life cycle toxicity test on the fathead minnow (Pimephales promelas) 2013/1311402
Guidelines:	OECD 210, OECD 229, EPA 890.1350
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The chronic toxicity of iprodione to fathead minnow (*Pimephales promelas*) was evaluated in a 56 days partial life-cycle test under flow-through conditions. The test was started with the exposure of 7 months old reproductively active fish in 5 replicates per concentration with 2 male and 4 female fish in each replicate. On day 22, the F1-generation was started using viable eggs from the corresponding F0 test groups with 4 replicates per test group and 25 eggs in each replicate. Nominal concentrations of iprodione were: 0 (control), 0.0033, 0.010, 0.030, 0.090 and 0.270 mg iprodione/L, corresponding to overall geometric mean measured concentrations of 0, 0.0029, 0.0084, 0.0267, 0.0731 and 0.238 mg iprodione/L and to 0, 0.0029, 0.0109, 0.0310, 0.0870 and 0.2771 mg iprodione plus the transient metabolite RP 35606/L. In this study survival and growth of fathead minnow was observed over two generations. The reproduction of the F0-generation was monitored by recording egg-laying and fertility. The gonads of F0-generation fish from the control and the highest treatment group underwent histological examination.

The following biological results are based on nominal concentrations. There were no observable sublethal effects in the F0- and F1-generation and no adverse treatment-related effects on histopathology in F0-generation fish in any of test item concentrations tested. No significant effects on survival and growth in F0-generation fish were detected over the 32 day exposure. Reduced fecundity, but not fertility, was observed in F0-generation fish in the highest test item concentration. There was no adverse treatment-related effect on hatching success, time to hatch, or survival in the F1-generation. The F1-generation growth was statistically significantly reduced compared to the control at the highest test item concentration. However, in fish life cycle studies, the F1 generation necessarily originates from the corresponding F0 test group and not from a homogenous pool of embryos, therefore based on the design of the study a genetic factor (unrelated to test substance) cannot be excluded as a contributing factor to the observed F1-generation responses.

In a partial life cycle test conducted under flow through conditions, fathead minnows were exposed to iprodione over a period of 56 days. The overall NOEC identified in this study was 0.090 mg iprodione/L based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Iprodione (BAS 610 F, Reg. No. 101 169), batch no. COD-001260, purity: 97.8 ± 1.0%.

B. STUDY DESIGN

Test species: Fathead minnow (*Pimephales promelas*); the F0 generation was started with 7 months old reproductively active adult fish; source: parental from Osage Catfisheries, Osage Beach (MO), USA.

Test design: Flow-through system (56 days); 5 test concentrations plus control. F0-generation: 5 replicates per treatment with 2 male and 4 female fish in each, exposure over 32 days. F1-generation: started at day 22 with 4 replicates per concentration using intact viable eggs (25 eggs per replicate) from the corresponding F0 test groups, exposure over 33 days. Survival and growth was observed over two generations; reproduction of the F0-generation was monitored by recording egg-laying and fertility; histological examination of gonads of F0-generation fish from the control and the highest test item concentration; daily observation of signs of effects and abnormalities.

Endpoints: NOEC values based on survival, growth, reproduction, signs of effects and abnormalities and histopathology of gonads.

Test concentrations: Control, 0.0033, 0.010, 0.030, 0.090 and 0.270 mg iprodione/L (nominal), corresponding to overall geometric mean measured concentrations of 0, 0.0029, 0.0084, 0.0267, 0.0731 and 0.238 mg iprodione/L and to 0, 0.0029, 0.0109, 0.0310, 0.0870 and 0.2771 mg iprodione plus RP 35606/L.

Test conditions:	Test vessels: F0-generation: 9 L stainless steel aquaria with two stainless steel spawning tiles, partly covered with opaque lids; F1-generation: 1.7 L cylindrical glass vessels for exposure as fertilized eggs and larvae, 9 days post-hatch juveniles were transferred to 9-L stainless steel aquaria. Dilution water: aerated, non-chlorinated, filtered drinking water (mixed with deionized water); temperature 25 ± 1 °C; conductivity 335 - 342 µS; acid capacity 1.04 - 1.06 mmol/L; total organic carbon: 2.0 mg/L. F0-generation: pH 6.9 - 7.3; oxygen content 6.2 - 7.6 mg/L; total hardness: 1.07 - 1.11 mmol/L. F1-generation: pH 6.9 - 7.3; oxygen content 5.9 - 8.3 mg/L; total hardness: 1.09 - 1.13 mmol/L. Flow rate: 1.7 L glass test vessels (embryo/larval exposure): > 0.5 L/hour (> 5 fold volume exchange); 9-L stainless steel aquaria: ≥ 1.9 L/hour (≥ 5 fold volume exchange). Photoperiod: 16 h light: 8 h dark; F0-generation: 74- 161 Lux; F1-generation: 58 - 213 Lux. Feeding: commercial fish diet (twice daily) and live brine shrimp nauplii (*Artemia salina);* feeding of F1-generation from day 6 on. Aeration: F0: slight aeration from test initiation on; F1: slight aeration from day 17 on.
Analytics:	The test item concentrations were analyzed using a HPLC method with MS detection. The analytical measurements included the transient metabolite RP 35606 (Reg. No. 5079626), which can revert back to parent.
Statistics:	Descriptive statistics; Williams test or Jonckheere-Terpstra test for determination of the NOEC values ($p \leq 0.01$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentrations was conducted in all treatments of the F0 generation on days 0, 5, 12, 20, 25 and 32. For the F1 generation, analytical measurements were conducted on days 5, 13, 19, 24 and 33. The overall geometric mean measured concentrations of the transient metabolite RP 35606 in each treatment were < LoD (limit of detection), < LoQ (limit of quantification), 0.0025, 0.0044, 0.0140 and 0.0390 mg/L. Overall geometric mean measured concentrations of iprodione over the entire exposure period ranged from 81% to 89% of nominal, whereas overall geometric mean measured concentrations of iprodione plus the transient metabolite RP 35606 ranged from 88% to 109% of nominal. The geometric mean measured concentrations are considered an accurate representation of the actual exposure concentrations over the course of the test. The following biological results are based on nominal concentrations. Additionally, the endpoints based on overall geometric mean measured concentrations of iprodione and on overall geometric mean measured concentrations of iprodione plus the transient metabolite RP 35606 are provided.

Biological results: There were no observable sublethal effects in the F0- and F1-generation and no adverse treatment-related effects on histopathology in F0-generation fish in any of test item concentrations tested. No significant effects on survival and growth in F0-generation fish were detected over the 32 day exposure. Reduced fecundity, but not fertility, was observed in F0-generation fish in the highest test item concentration (Jonckheere-Terpstra test, $p \leq 0.01$). There was no adverse treatment-related effect on hatching success, time to hatch, or survival in the F1-generation. The F1-generation growth was statistically significantly reduced compared to the control at the highest test item concentration (Williams test, $p \leq 0.01$). However, in fish life cycle studies, the F1 generation necessarily originates from the corresponding F0 test group and not from a homogenous pool of embryos, therefore based on the design of the study a genetic factor (unrelated to test substance) cannot be excluded as a contributing factor to the observed F1-generation responses. The results are summarized in Table 8.2.3-2.

Table 8.2.3-2: Chronic toxicity (partial life cycle, 56 days) of iprodione on fathead minnow

Concentration [mg a.s./L] (nominal)			**Control**	**0.0033**	**0.010**	**0.030**	**0.090**	**0.270**
Concentration [mg a.s./L] (overall geometric mean measured iprodione)			**--**	**0.0029**	**0.0084**	**0.0267**	**0.0731**	**0.238**
Concentration [mg a.s./L] (overall geometric mean measured iprodione + RP 35606)			**--**	**0.0029**	**0.0109**	**0.0310**	**0.0870**	**0.2771**
Survival	F0	start of exposure - sacrifice [%]	100	100	100	100	100	100
	F1	hatching success [%]	95	97	97	97	97	98
		larval survival to end of swim up [%]	100	100	100	100	100	99
		end of swim up - sacrifice [%]	100	98	97	98	98	100
Growth	F0	male length at sacrifice [cm]	6.0	6.1	6.0	6.2	6.3	6.1
		female length at sacrifice [cm]	4.9	4.9	5.0	4.9	5.0	5.0
		male wet weight at sacrifice [g]	2.92	3.11	3.19	3.36	3.45	3.32
		female wet weight at sacrifice [g]	1.30	1.35	1.35	1.35	1.35	1.45
	F1	length at sacrifice [cm]	2.9	2.9	2.9	3.0	2.9	**2.8 ***
		wet weight at sacrifice [mg]	251	239	240	248	242	**212 ***
Reproduction	F0	fertility [%]	99.7	99.6	99.7	99.7	99.7	99.7
		fecundity [total eggs/female reproductive day]	25.4	24.6	22.3	24.6	24.1	**13.7** #

	Endpoints [mg iprodione/L]
Overall NOEC (56 days) (nominal)	**0.090**
Overall NOEC (56 days) (overall geometric mean measured iprodione)	0.0731
Overall NOEC (56 days) (overall geometric mean measured iprodione + RP 35606)	0.0869

Deviations which are considered to be substance-related are printed **bold**.
* Statistically significant differences compared to the control (Williams test, $p \leq 0.01$)
\# Statistically significant differences compared to the control (Jonckheere-Terpstra test, $p \leq 0.01$)

III. CONCLUSION

In a partial life cycle test conducted under flow through conditions, fathead minnows were exposed to iprodione over a period of 56 days. The overall NOEC identified in this study was 0.090 mg iprodione/L based on nominal concentrations.

The following Amphibian Metamorphosis Assay performed with iprodione on *Xenopus laevis* was performed as US EPA EDSP Tier 1 screening study for US registration. It is not required for registration in the EU and thus, it has not been evaluated previously on EU level. Nevertheless, the summary of this study is included below for the sake of completeness.

Report:	CA 8.2.3/3 [redacted]., 2012a Iprodione - Amphibian metamorphosis assay with African clawed frog (Xenopus laevis) following OPPTS test guideline 890.1100 and OECD test guideline No. 231 2012/1364404
Guidelines:	EPA 890.1100, OECD 231
GLP:	yes (certified by United States Environmental Protection Agency) [see 2014/1049126 Shearer K.S. 2014 c]

Executive Summary

The potential endocrine activity of iprodione in African Clawed Frog (*Xenopus laevis*) tadpoles was evaluated in a 21-day Amphibian Metamorphosis Assay (AMA) under flow-through conditions. The AMA is a screening assay intended to empirically identify substances which may interfere with the normal function of the hypothalamic-pituitary-thyroid (HPT) axis. Frogs were exposed to a dilution water control and to nominal test item concentrations of 0.0070, 0.070 and 0.70 mg iprodione/L (equivalent to mean measured concentrations of 0.0054, 0.066 and 0.61 mg a.s./L). The evaluated endpoints were mortality, developmental stage, snout-vent length (SVL), hind limb length, whole body weight, toxic signs (behavior and other sublethal effects) and thyroid gland histology.

The biological results are based on mean measured concentrations of iprodione. Exposure of tadpoles to iprodione at concentrations of up to an including 0.70 mg a.s./L was not found to significantly increase or decrease any of the evaluated biological endpoints (*i.e.* survival, whole body weight, SVL, hind limb length) throughout the 21-day study. Furthermore, no treatment-related toxic signs (abnormal behavior and other sublethal effects) were observed. Asynchronous development or significant acceleration or delay of median developmental stage was not observed for any treatment level. Effects on thyroid gland histopathology were not observed at any treatment level either and no treatment related lesions were identified.

In an Amphibian Metamorphosis Assay with African Clawed Frog (*Xenopus laevis*) tadpoles, the overall NOEC (21 d) for iprodione was determined to be ≥0.70 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Iprodione (BAS 610 F, Reg. no. 101 169), batch no. SIN0000217 (AE F062470-01-08), purity: 98.1%.

B. STUDY DESIGN

Test species: South African Clawed Frog (*Xenopus laevis*) tadpoles, NF stage 51 and 12 days old post-fertilization at exposure initiation; all tadpoles used in the test originated from a single spawn; source: tadpoles originate from adult brood stocks maintained in-house; brood stock was originally obtained from Masco, Fort Atkinson, Wisconsin, USA.

Test design: Flow-through system (21 d); 3 test item concentrations plus a dilution water control, 4 replicates per treatment with 20 tadpoles (day 12 post-fertilization) in each. The exposure system consisted of an intermittent flow proportional diluter, a temperature-controlled water bath and a set of 16 exposure aquaria. Staging on tadpoles was performed at test initiation based on hind limb morphology which is the primary developmental landmark for staging 51 NF tadpoles. During the exposure period, survival and toxic signs (appearance and abnormal behavior) were daily assessed in all treatments.
On day 7, five tadpoles were randomly selected from each test vessel for determination of the growth metrics. At test termination (day 21) the remaining tadpoles were sacrificed and the animal weight and lengths (snout-vent length and hind limb length) was measured and the developmental stages were determined. For thyroid gland histology a total of five tadpoles were selected from each replicate test concentration.

Endpoints: NOEC values based on mortality, toxic signs (behavioral and sub-lethal observations), developmental stage, snout-vent length (SVL), hind limb length, hind limb length (normalized by SVL), body weight and thyroid gland histology

Test concentrations: Control (dilution water), 0.0070, 0.070 and 0.70 mg iprodione/L (nominal); equivalent to mean measured concentrations of 0.0054, 0.066 and 0.61 mg iprodione/L.

Test conditions:	2.5-gallon glass aquaria (30 x 15 x 20 cm) positioned in water baths; test volume: 6.5 L; dilution water: FETAX solution (according to ASTM, 2004); flow rate: 250 mL/per cycle/aquarium resulting in 7 volume exchanges/aquarium/24 h); water temperature: 21 °C - 23 °C; pH: 7.3 - 8.2; oxygen saturation: 53% - 88%; total hardness: 160 - 180 mg $CaCO_3$/L; conductivity: 1800 - 2000 µS/cm; alkalinity: 78 - 82 mg $CaCO_3$/L; light intensity: approx. 900 - 1100 lux; photoperiod: 12 h light : 12 h dark; feeding: tadpoles were fed Xenopus Express Tadpole Food (Xenopus Express, Brooksville, Florida) as a solution in dilution water twice a day in increasing amounts according to their age.
Analytics:	Analytical verification of iprodione concentrations was conducted using an HPLC-method with UV detection.
Statistics:	Descriptive statistics; Fisher's Exact test with Bonferroni-Holm adjustment for survival data; Jonckheere-Terpstra Step-Down Test for developmental stage data; Jonckheere-Terpstra Step-Down Test or Dunnett's Multiple Comparison Test for weight and length data.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical measurements of iprodione concentrations in the exposure solutions of each treatment were conducted at test initiation, on days 7 and 14 and at test termination. The mean measured concentrations of iprodione measured in the application solutions taken at test initiation ranged from 80.0% to 108.6% of nominal concentrations. 7 days after application, measured water concentrations of iprodione in all test item treatments were between 81.4% and 104.3% of nominal and between 67.1% and 81.4% of nominal on day 14. At exposure termination on day 21, the measured concentrations ranged from 71.4% to 87.1% of nominal. The mean recovery rates over the whole study period were 78%, 94% and 87% of nominal in the test concentrations of 0.0054, 0.066 and 0.61, respectively. The following biological results are based on mean measured concentration.

Biological results:

Biological observations: During the study, a varying number of tadpoles in the control and the test item treatments were observed to be deformed (*e.g.,* spinal curvature). However, the incidence of spinal deformities did not appear to have a dose-response relationship and did not impact any endpoint determined in this assay.

Larval survival: Following 21 days of exposure, the percent survival in the control averaged 99% and the percent survival rate in all test item treatments averaged 100%. No statistically significant differences were observed compared to the control (Fisher`s Exact test with Bonferroni-Holm adjustment).

Whole body wet weight: No statistical significant effects on tadpole whole body weight, snout-vent-length (SVL), himb limb length and hind limb length normalized by SVL compared to the control were detected in any test item treatment group, neither after 7 nor 21 days of exposure to iprodione (Dunnett`s Multiple Comparison Test or Jonckheere-Terpstra Step-Down Test).

Developmental stage: At termination of exposure, 4% of the animals in the control were observed to be late stage (developmental stage > 60NF). Late stage was observed in 5.0%, 5.0% and 3.0% of the tadpoles exposed to the 0.0054, 0.066 and 0.61 mg a.s./L treatment levels, respectively. No significant effects on tadpole developmental stages were observed in any test item concentration after 7 and 21 days of exposure when compared to the control (Jonckheere-Terpstra Step-Down Test).

Thyroid histology: No treatment-related effects on thyroid gland histology were identified at any treatment level. Mild follicular cell hypertrophy or mild follicular cell hyperplasia (35% to 40% of the animals) and mild thyroid gland hypertrophy (5 to 15% of the animals) was diagnosed throughout the study in the controls and all treatment levels tested. The similarity in incidence and severity across treatments indicate these are not iprodione related findings, but a reflection of normal developmental variation.

The results are summarized in Table 8.2.3-3.

Table 8.2.3-3: Chronic toxicity and endocrine activity of iprodione to African Clawed Frog (*Xenopus laevis*) in an Amphibian Metamorphosis Assay (21 d)

Concentration [mg a.s./L] (nominal)	**Control**	**0.0070**	**0.070**	**0.70**
Concentration [mg a.s./L] (mean measured)	**Control**	**0.0054**	**0.066**	**0.61**
Survival (21 d) [%]	99	100	100	100
Whole body weight (7 d) [g] [1)]	0.41	0.44	0.45	0.40
Whole body weight (21 d) [g] [2)]	1.65	1.62	1.62	1.55
Snout-Vent Length (7 d) [mm] [1)]	18.23	18.31	18.98	18.04
Snout-Vent Length (21 d) [mm] [1)]	28.37	28.49	28.26	28.40
Hind Limb Length (7 d) [mm] [1), 3)]	2.15 (0.118)	2.12 (0.115)	2.20 (0.116)	2.10 (0.116)
Hind Limb Length (21 d) [mm] [1), 3)]	16.91 (0.60)	17.60 (0.62)	16.45 (0.58)	15.82 (0.56)
Median developmental stage (7 d)	54	54	54	54
Median developmental stage (21 d)	60	60	59	59
Mean spinal deformities (7 d) [%]	40	25	0	5
Mean spinal deformities (21 d)[%]	41	30	37	20
Mean spinal deformities (total) [%]	41	29	28	16
Thyroid gland histology (21 d)	NSF	NSF	NSF	NSF
	Endpoints [mg a.s./L] (mean measured)			
$NOEC_{overall}$	**≥ 0.70**			

NSF = no significant findings
1) A monotonic trend was not observed with these data and thus the statistical analyses were applied to replicate means.
2) A monotonic trend was observed with these data and thus the statistical analyses were applied to replicate medians.
3) Values in brackets are normalized by Snout-Vent-Length.

III. CONCLUSION

In an Amphibian Metamorphosis Assay with African Clawed Frog (*Xenopus laevis*) tadpoles, the overall NOEC (21 d) for iprodione was determined to be ≥0.70 mg a.s./L, based on mean measured concentrations.

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 RP 36221 is provided in support of the aquatic risk assessment and has not been evaluated previously. The study is required, due to new data requirements.

Report:	CA 8.2.4.1/1 Swierkot A., 2013c Reg. No. 5079618 (metabolite of BAS 610 F, Iprodione, RP036221) - Daphnia magna, acute immobilization test 2013/1191247
Guidelines:	OECD 202 (2004)
GLP:	yes (certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Executive Summary

In a static acute toxicity laboratory study, water flea neonates were exposed to RP 36221 (metabolite of iprodione) at geometric mean measured concentrations of 0 (control), 0.038, 0.080, 0.165, 0.321 and 0.664 mg/L in 4 replicates per concentration containing 5 daphnids each. Daphnids were observed for immobility 24 hours and 48 hours after start of exposure.

The biological results are based on geometric mean measured concentrations. After 48 h of exposure, no immobility of daphnids was observed in the control and at the three lowest tested concentrations, whereas 15% of the daphnids were immobile at 0.321 mg/L. At the highest test item concentration all daphnids were immobile after 48 hours of exposure. Statistically significant effects on mobility of daphnids were detected at the highest test item concentrations of 0.664 mg RP036221/L.

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of RP 36221 (metabolite of iprodione) was determined to be 0.364 mg/L based on geometric mean measured concentrations. The NOEC was 0.321 mg/L (geometric mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: RP 36221 (Reg. No. 5 079 618, metabolite of iprodione), batch no. L80-172, purity: 98.4% ± 1%.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates from in-house culture, < 24 hours old at test initiation, not first brood progeny.

Test design: Static system (48 hours); 5 test item concentrations plus a control, 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.

Endpoints: EC_{50} and NOEC based on immobility of daphnids.

Test concentrations: Control, 0.038, 0.080, 0.165, 0.321 and 0.664 mg RP036221/L (geometric mean measured); due to the poor solubility of the test item in water, the test item concentrations were obtained using Water Accommodated Fractions (WAFs); the WAF of a loading of 100 mg RP036221/L was used as highest test item concentration (stock solution); the lower concentrations were prepared by sequential dilution with the test medium in order to prepare a series of five test item concentrations with a spacing factor of 2.0 (*i.e.* 16-fold, 8-fold, 4-fold and 2-fold dilutions of the WAF of the highest test concentration were prepared).

Test conditions: 150 mL glass beakers, test volume 100 mL, dilution water: “M7” (Elendt medium); temperature: 19.4 °C - 20.1 °C; pH 7.62 - 7.73 at test initiation and pH 7.60 - 7.68 at test termination; oxygen content: 8.1 mg/L - 8.5 mg/L at test initiation and 7.9 mg/L - 8.3 mg/L at test termination; photoperiod: 16 hours light : 8 hours dark; no feeding and no aeration.

Analytics: Analytical verification of test item concentrations was conducted using a LC-method with DAD-detection.

Statistics: Descriptive statistics. Fisher`s Exact Binomial Test with Bonferroni Correction for determination of the NOEC; probit analysis for determination of the EC_{50} value.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at the beginning and at the end of the test. At test initiation, the following initial test item concentrations were measured: 0.041, 0.086, 0.175, 0.343, 0.696 mg RP036221/L. Measured values at test termination ranged from 87.21% to 90.95% of initial concentrations. Therefore, the concentrations of the test item were stable under test conditions. Because of the poor solubility of the test item, endpoint values are based on geometric mean measured test item concentrations.

Biological results: After 48 h of exposure, no immobility of daphnids was observed in the control and at the three lowest tested concentrations, whereas 15% of the daphnids were immobile at 0.321 mg/L. At the highest test item concentration all daphnids were immobile after 48 hours of exposure. Statistically significant effects on mobility of daphnids were detected at the highest test item concentrations of 0.664 mg RP036221/L (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$). For results see Table 8.2.4.1-1.

Table 8.2.4.1-1: Effect of RP 36221 (metabolite of iprodione) on *Daphnia magna* immobility

Concentration (using WAFs)	**Control**	**16-fold diluted**	**8-fold diluted**	**4-fold diluted**	**2-fold diluted**	**stock solution (WAF of a loading of 100 mg/L)**
Concentration [mg/L] (geometric mean measured)	**--**	**0.038**	**0.080**	**0.165**	**0.321**	**0.664**
Immobility (24 h) [%]	0	0	0	0	0	15
Immobility (48 h) [%]	0	0	0	0	15	100 *
	Endpoints [mg RP036221/L] (geometric mean measured)					
EC_{50} (48 h)	0.364					
NOEC (48 h)	0.321					

WAF = Water Accommodated Fraction

* Statistically significantly different compared to the control (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$)

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of RP 36221 (metabolite of iprodione) was determined to be 0.364 mg/L based on geometric mean measured concentrations. The NOEC was 0.321 mg/L (geometric mean measured).

The following acute toxicity study on *Daphnia magna* performed with the metabolite RP 25040 is provided in support of the aquatic risk assessment and has not been evaluated previously. The study is required, due to new data requirements.

Report:	CA 8.2.4.1/2 Swierkot A., 2013a Reg.No. 207099 (metabolite of BAS 610 F, Iprodione, RP025040) Daphnia magna, acute immobilization test 2013/1000223
Guidelines:	OECD 202 (2004)
GLP:	yes (certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Executive Summary

In a 48-hour static acute toxicity laboratory study, water flea neonates were exposed to RP 25040 (metabolite of iprodione) at nominal concentrations of 0 (control), 6.25, 12.5, 25.0, 50.0 and 100.0 mg/L in 4 replicates per concentration, 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. After 48 hours of exposure, no immobility of daphnids was observed in the control and at test item concentrations of up to and including 50.0 mg/L, whereas 100% immobility occurred at the highest test item concentration of 100.0 mg/L. The effects on daphnids detected at 100.0 mg/L were statistically significantly different compared to the control.

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of RP 25040 was 70.54 mg/L based on nominal concentrations. The NOEC was determined to be 50.0 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: RP 25040 (Reg. No. 207 099, metabolite of iprodione), batch no. TV2840/F; purity: 99.6% (± 1%).

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates collected from the laboratory culture cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Aquatic Toxicology; < 24 hours old at test initiation.

Test design: Static system (48 hours), 5 test item concentrations plus control, 4 replicates with 5 daphnids in each treatment; assessment of immobility after 24 and 48 hours.

Endpoints: NOEC, EC_{50} based on immobility of daphnids.

Test concentrations: Control, 6.25, 12.5, 25.0, 50.0 and 100.0 mg RP 25040/L (nominal).

Test conditions: 150 mL glass vessels, test volume 100 mL, dilution water "M7" (Elendt M7 medium); pH 7.55 - 7.78; oxygen content: 7.7 mg/L - 8.8 mg/L; temperature: 19.7 - 21.3ºC; photoperiod: 16 h light: 8 h dark, no feeding, no aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with DAD.

Statistics: Descriptive statistics; probit analysis for determination of the EC_{50}; Fisher`s Exact Binominal Test with Bonferroni Correction for determination of the NOEC ($\alpha = 0.05$)

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 analyzed contents of RP 25040 ranged from 87.4% to 93.8% of nominal concentrations at test initiation and from 70.6% to 76.1% of nominal at test termination. As the initially measured concentrations confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: The biological results are based on nominal concentrations. After 48 hours of exposure, no immobility of daphnids was observed in the control and at test item concentrations of up to and including 50.0 mg/L, whereas 100% immobility occurred at the highest test item concentration of 100.0 mg/L. The effects on daphnids detected at 100.0 mg/L were statistically significantly different compared to the control (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$). For results see Table 8.2.4.1-2.

Table 8.2.4.1-2: Effects of RP 25040 on *Daphnia magna* immobility

Concentration [mg/L] (nominal)	**Control**	**6.25**	**12.5**	**25.0**	**50.0**	**100.0**
Immobility (24 h) [%]	0	0	0	0	0	100
Immobility (48 h) [%]	0	0	0	0	0	100 *
Endpoints [mg RP 25040/L] (nominal)						
EC_{50} (48 h)	70.54					
NOEC (48 h)	50.0					

* Statistically significantly different compared to the control (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$)

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of RP 25040 was 70.54 mg/L based on nominal concentrations. The NOEC was determined to be 50.0 mg/L (nominal).

The following acute toxicity study on *Daphnia magna* performed with the metabolite RP 32596 is provided in support of the aquatic risk assessment and has not been evaluated previously. The study is required, due to new data requirements.

Report:	CA 8.2.4.1/3 Rzodeczko H., 2013b Reg.No. 85831 (metabolite of BAS 610 F, Iprodione, RP032596) - Daphnia magna acute immobilization test 2013/1000225
Guidelines:	OECD 202 (2004)
GLP:	yes (certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Executive Summary

In a 48-hour static acute toxicity laboratory study, water flea neonates were exposed to RP 32596 (metabolite of iprodione) at nominal concentrations of 0 (control), 0.18, 0.40, 0.88, 1.94, 4.27, 9.39, 20.66, 45.45 and 100.0 mg/L in 4 replicates per concentration, 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. After 48 hours of exposure, no immobility of daphnids was observed in the control and at the test item concentrations of up to and including 0.40 mg/L, whereas 5% immobility occurred at 0.88 mg/L. At 1.94 mg/L and all higher concentrations, 100% immobility was detected after 48 hours of exposure.

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of RP 32596 was 1.26 mg/L based on nominal concentrations. The NOEC was determined to be 0.88 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: RP 32596 (Reg. No. 85 831, metabolite of iprodione), batch no. L33-79F; purity: 99.8%.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates collected from the laboratory culture cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Aquatic Toxicology; < 24 hours old at test initiation.

Test design: Static system (48 hours), 9 test concentrations plus control, 4 replicates with 5 daphnids in each treatment; assessment of immobility after 24 and 48 hours.

Endpoints: NOEC, EC_{50} based on immobility of daphnids.

Test concentrations: Control, 0.18, 0.40, 0.88, 1.94, 4.27, 9.39, 20.66, 45.45 and 100.0 mg RP 32596/L (nominal).

Test conditions: 150 mL glass vessels, test volume 100 mL, dilution water "M7" (Elendt M7 medium); pH 7.51 - 8.05; oxygen content: 8.1 mg/L - 8.3 mg/L; temperature: 21.4 - 22.8ºC; photoperiod: 16 h light: 8 h dark, no feeding, no aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with DAD-detection.

Statistics: Descriptive statistics; probit analysis for determination of the EC_{50}; Fisher`s Exact Binominal Test with Bonferroni Correction for determination of the NOEC ($\alpha = 0.05$).

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 analyzed contents of RP 32596 ranged from 91.0% to 104.3% of nominal concentrations at test initiation and from 87.2% to 102.2% of nominal at test termination. As the measured concentrations confirmed the correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: After 48 hours of exposure, no immobility of daphnids was observed in the control and at the test item concentrations of up to and including 0.40 mg/L, whereas 5% immobility occurred at 0.88 mg/L. At 1.94 mg/L and all higher concentrations, 100% immobility was detected after 48 hours of exposure. For results see Table 8.2.4.1-3.

Table 8.2.4.1-3: Effects of RP 32596 on *Daphnia magna* immobility

Concentration [mg/L] (nominal)	**Control**	**0.18**	**0.40**	**0.88**	**1.94**	**4.27**	**9.39**	**20.66**	**45.45**	**100.0**
Immobility (24 h) [%]	0	0	0	0	80	100	100	100	100	100
Immobility (48 h) [%]	0	0	0	5	100	100	100	100	100	100
Endpoints [mg RP 32596/L] (nominal)										
EC_{50} (48 h)	1.26 (95% confidence limits: 1.09 - 1.46)									
NOEC (48 h)	0.88									

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of RP 32596 was 1.26 mg/L based on nominal concentrations. The NOEC was determined to be 0.88 mg/L (nominal).

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

The following acute toxicity study on the marine saltwater mysid *Americamysis bahia* (former name: *Mysidopsis bahia*) performed with the active substance iprodione is not required for registration in the EU. However, the study was conducted due to U.S. data requirements and it is provided for the sake of completeness. It has not been evaluated previously on EU level.
The 48-h LC_{50} obtained in the 96 h study is used for the risk assessment of iprodione 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. This harmonizes the duration of acute toxicity tests among aquatic arthropods.

Report:	CA 8.2.4.2/1 Surprenant D.C., 1987a Acute toxicity of Iprodione technical to mysid shrimp (Mysidopsis bahia) under flow-through conditions C022006
Guidelines:	EPA 72-3
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In an acute toxicity laboratory study under flow-through conditions, saltwater mysids were exposed to a dilution water control, a solvent control and to nominal concentrations of 0.45, 0.69, 1.1, 1.6 and 2.5 mg iprodione/L (corresponding to mean measured concentrations of 0.25, 0.27, 0.36, 0.57 and 0.97 mg/L) in groups of 10 animals per glass aquaria (two test chambers per aquarium with 5 animals in each) containing 19 L water with 2 replicates per concentration. The mysids were observed for survival and symptoms of toxicity 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations. After 96 hours of exposure 10% and 5% mortality were observed in the control and solvent control, respectively. Mortality rates of 15%, 15%, 15%, 30% and 75% were observed in the concentrations of 0.25, 0.27, 0.36, 0.57 and 0.97 mg iprodione/L after 96 hours, respectively. No other toxic effects were observed in the controls and at 0.27 mg iprodione/L whereas signs of toxicity among mysids appeared in all other test item concentrations, including erratic swimming, lying/swimming at the bottom; dark pigmentation and lethargy.

In a flow-through acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (96 h) for iprodione was determined to be 0.68 mg/L based on mean measured concentrations. The NOEC (96 h) was < 0.25 mg/L (mean measured). The LC_{50} (48 h) for iprodione was determined to be > 0.97 mg/L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 610 F (Reg. No. 101 169), lot no. 67-64-1, purity: 100%.

B. STUDY DESIGN

Test species: Saltwater mysid (*Mysidopsis bahia;* nowadays called: *Americamysis bahia*), juveniles, age: less than 24 hours old; source: in-house cultures.

Test design: Flow-through system (96 hours); 10 mysids per test aquaria (two test chamber per aquarium each containing 5 mysids), 2 replicate aquaria per concentration; assessment of mortality and symptoms of toxicity 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50} (48 h and 96 h), NOEC (96 h), mortality and sub-lethal effects.

Test concentrations: Control (dilution water), solvent control (0.066 mL/L acetone); 0.45, 0.69, 1.1, 1.6 and 2.5 mg iprodione/L (nominal); corresponding to mean measured concentrations of 0.25, 0.27, 0.36, 0.57 and 0.97 mg a.s./L.

Test conditions: Glass aquaria (39 x 20 x 25 cm) with two test chambers, test chambers: glass petri dishes (10 cm diameter, 2 cm depth) with a 15 cm high nylon screen collar (363 µm mesh size) attached; test volume 3.1 - 7.0 L, filtered natural seawater, solution exchange: 90% per 4.5 hours; salinity: 30‰; temperature: 24 °C - 25 °C; pH 7.7 - 7.8; oxygen content: 6.0 mg/L- 7.2 mg/L; photoperiod 16 h light : 8 h dark; light intensity: 30 - 80 foot-candles; juvenile mysids were fed with brine shrimps two times daily.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with variable wavelength detection.

Statistics: Descriptive statistics; probit analysis for calculation of LC_{50} (96 h).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at test initiation and at test termination. Mean measured concentrations for iprodione ranged from 38.6% to 48.9% of nominal concentrations at test initiation and from 26.8% to 62.2% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure 10% and 5% mortality were observed in the control and solvent control, respectively. Mortality rates of 15%, 15%, 15%, 30% and 75% were observed in the concentrations of 0.25, 0.27, 0.36, 0.57 and 0.97 mg iprodione/L after 96 hours, respectively. No other toxic effects were observed in the controls and at 0.27 mg iprodione/L whereas signs of toxicity among mysids appeared in all other test item concentrations, including erratic swimming, lying/swimming at the bottom; dark pigmentation and lethargy. The results are summarized in Table 8.2.4.2-1.

Table 8.2.4.2-1: Acute toxicity of iprodione to saltwater mysids (*Americamysis bahia*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.45**	**0.69**	**1.1**	**1.6**	**2.5**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.25**	**0.27**	**0.36**	**0.57**	**0.97**
Mortality [%] (48 h)	0	0	5	0	5	5	45
Mortality [%] (96 h)	10	5	15	15	15	30	75
Symptoms (96 h) *	none	none	A	none	A, B	A	B, C
Endpoints [mg iprodione/L] (mean measured)							
LC_{50} (96 h)	0.68 (95% confidence limits: 0.54 - 1.0)						
NOEC (96 h)	< 0.25						

* Symptoms after 96 hours: A = erratic swimming; B = lethargy, C = dark pigmentation

III. CONCLUSION

In a flow-through acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (96 h) for iprodione was determined to be 0.68 mg/L based on mean measured concentrations. The NOEC (96 h) was < 0.25 mg/L (mean measured). The LC_{50} (48 h) for iprodione was determined to be > 0.97 mg/L (mean measured).

The following acute toxicity study on eastern oyster *Crassostrea virginica* performed with the active substance iprodione is not required for registration in the EU. However, the study was conducted due to U.S. data requirements and it is provided for the sake of completeness. It has not been evaluated previously on EU level.

Report:	CA 8.2.4.2/2 Surprenant D.C., 1987b Acute toxicity of Iprodione technical to Eastern oysters (Crassostrea virginica) under flow-through conditions C021944
Guidelines:	EPA 72-3
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

A study was conducted to determine the effects of iprodione on the shell deposition of eastern oysters during a 96-hour exposure period under flow-through test conditions. The eastern oysters were exposed to a dilution water control, a solvent control and to nominal test item concentrations of 1.0, 1.7, 2.9, 4.8 and 8.0 mg iprodione/L (corresponding to mean measured concentrations of 1.0, 1.6, 2.3, 3.7 and 5.2 mg iprodione/L) in groups of 20 oysters in glass aquaria each containing 18 L seawater 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. After 48 hours, oysters exposed to test item concentrations of 2.3 mg/L and higher appeared to be siphoning and feeding less than the control oysters. However, no mortalities occurred during the 96-hour exposure period in both control groups and in all test item treatment groups. Statistically significant inhibition of shell growth compared to the pooled control was observed at concentrations of 1.6 mg iprodione/L and all higher concentrations.

In a flow-through acute toxicity study with eastern oysters (*Crassostrea virginica*), the EC_{50} (96 h) for iprodione was 2.3 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 1.0 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Iprodione (BAS 610 F; Reg. no.: 101 169), lot no. 84-251-01; purity: 100%.

B. STUDY DESIGN

Test species: Eastern oysters (*Crassostrea virginica*), mean valve height of 39 mm ± 5 mm; source: Aquacultural Research Corporation, Dennis, Massachusetts, 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 20 oysters per replicate test chamber (giving a total number of 40 animals per treatment); daily assessment of mortality and symptoms of toxicity; measurements of shell deposition 96 hours after start of exposure.

Endpoints: EC_{50} (96 h) and NOEC for shell growth inhibition; mortality and symptoms of toxicity.

Test concentrations: Control (dilution water: unfiltered seawater), solvent control (0.1 mL/L acetone); 1.0, 1.7, 2.9, 4.8 and 8.0 mg iprodione/L (nominal), corresponding to mean measured concentrations of 1.0, 1.6, 2.3, 3.7 and 5.2 mg a.s./L.

Test conditions: 54 L glass aquaria (60 cm x 30 cm x 30 cm), test volume approximately 18 L, natural unfiltered seawater, flow rate: 75 mL/minute resulting in 6 volume exchanges/aquarium/24 h; salinity: 27‰ - 31‰; temperature: 18.0 °C - 19.5 °C; pH 7.7 - 7.9; oxygen content: 6.7 mg/L - 8.2 mg/L; photoperiod 16 h light : 8 h dark; supplementary diet for oysters: suspension of marine microalgae at approx. 10^5 cells/aquarium three times daily.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with variable wavelength detection.

Statistics: Descriptive statistics; linear regression analysis for calculation of EC_{50} (96 h), ANOVA followed by Williams' test for shell deposition data ($\alpha = 0.05$).

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 iprodione ranged from 53.1% to 98.5% of nominal concentrations at test initiation and from 77.5% to 110.0% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 48 hours, oysters exposed to test item concentrations of 2.3 mg/L and higher appeared to be siphoning and feeding less than the control oysters. However, no mortalities occurred during the 96-hour exposure period in both control groups and in all test item treatment groups. Statistically significant inhibition (Williams' test, $\alpha = 0.05$) of shell growth compared to the pooled control was observed at concentrations of 1.6 mg iprodione/L and all higher concentrations. The results are summarized in Table 8.2.4.2-2.

Table 8.2.4.2-2: Acute toxicity (96 h) of iprodione to eastern oysters (*Crassostrea virginica*)

Concentration [mg a.s./L] (nominal)	**1.0**	**1.7**	**2.9**	**4.8**	**8.0**
Concentration [mg a.s./L] (mean measured)	**1.0**	**1.6**	**2.3**	**3.7**	**5.2**
Shell growth inhibition after 96 h [%] [1)]	13	26 *	52 *	83 *	78 *
	Endpoints [mg iprodione/L] (mean measured)				
EC_{50} (96 h)	2.3 (95% confidence limits: 1.0 - 5.3)				
NOEC (96 h)	1.0				

* Statistically significant difference compared to pooled control (Williams' test, $\alpha = 0.05$).
1) Percent inhibition compared to the pooled control.

III. CONCLUSION

In a flow-through acute toxicity study with eastern oysters (*Crassostrea virginica*), the EC_{50} (96 h) for iprodione was 2.3 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 1.0 mg a.s./L (mean measured).

The following acute toxicity study on juvenile crayfish (*Procambarus simulans*) performed with the active substance iprodione is not required for registration in the EU. However, the study was conducted due to U.S. data requirements and it is provided for the sake of completeness. It has not been evaluated previously on EU level.

Report:	CA 8.2.4.2/3 McAllister W.A.,Bunch B., 1986b Dynamic acute toxicity of Iprodione technical to juvenile crayfish (Procambarus simulans, Faxon) C021999
Guidelines:	none
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The effects of iprodione on the mortality of juvenile crayfish were investigated in flow-through acute toxicity study over a 7-day exposure period. The crayfish were exposed to a solvent control and nominal test item concentrations of 0.30, 0.70, 1.2, 2.4 and 5.0 mg iprodione/L (corresponding to mean measured concentrations of 0.26, 0.46, 0.92, 1.8 and 4.1 mg a.s./L) in groups of 20 crayfish in glass aquaria containing 30 L test water per concentration. The animals were observed for survival, symptoms of toxicity, molting and cannibalism at test initiation and once every 24 hours during the exposure period.

The biological results are based on mean measured concentrations. Molts were observed throughout the control and in all test item treatments, with the greatest number of 7 molts in total noted in the highest test item concentration of 4.1 mg iprodione/L. After 7 days of exposure, no mortality occurred in the control and in any test item treatment group.

In a flow-through, acute toxicity study with juvenile crayfish (*Procambarus simulans*), the LC_{50} (7 d) for iprodione was > 4.1 mg a.s./L based on mean measured concentrations. The NOEC was determined to be ≥ 4.1 mg iprodione/L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Iprodione (BAS 610 F; Reg. no.: 101 169), lot no. 84-251-01, purity: 95%.

B. STUDY DESIGN

Test species: Freshwater crayfish (*Procambarus simulans*), juveniles, mean weight 0.88 ± 0.18 g, mean length 29 ± 2.1 mm; source: "Northup Fish Hatchery", Centralia, Missouri, USA.

Test design: Flow-through system (7 days); 5 test item concentrations plus a solvent control, 1 replicate for each test item concentration and the solvent control; 20 crayfish per test aquaria; assessment of mortality, symptoms of toxicity, molting and cannibalism at test initiation and once every 24 hours during the exposure period. The length and weight of the test animals were measured at test initiation and, in the control, at test end.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Solvent control (0.1 mL/L acetone), 0.30, 0.70, 1.2, 2.4 and 5.0 mg iprodione/L (nominal), corresponding to mean measured concentrations of 0.26, 0.46, 0.92, 1.8 and 4.1 mg a.s./L.

Test conditions: Glass aquaria, test volume 30 L, dilution water: aerated well water, flow rate: 130 mL/minute/aquarium resulting in approximately 6 volume exchanges/aquarium/24 h; temperature: 21 °C - 22 °C; pH 8.0 - 8.2; oxygen content: 6.6 mg/L - 9.3 mg/L.

Analytics: Analytical verification of test item concentrations was conducted using a gas liquid chromatography-method with electron capture detection.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at test initiation, on day 4 and at test termination. Measured concentrations for iprodione ranged from 64.3% to 86.7% at test initiation, from 62.9% to 83.3% at day 4 and from 71.4% to 93.3% at test termination. The discrepancy between nominal and measured test item concentrations was attributed to the limited solubility of iprodione in the test dilution water. The following biological results are based on mean measured concentrations.

Biological results: Molts were observed throughout the control and in all test item treatments, with the greatest number of 7 molts in total noted in the highest test item concentration of 4.1 mg iprodione/L. After 7 days of exposure, no mortality occurred in the control and in any test item treatment group. The results are summarized in Table 8.2.4.1-3.

Table 8.2.4.2-3: Acute toxicity (7 d) of iprodione to juvenile crayfish (*Procambarus simulans*)

Concentration [mg a.s./L] (nominal)	**Solvent control**	**0.30**	**0.70**	**1.2**	**2.4**	**5.0**
Concentration [mg a.s./L] (mean measured)	**< 0.040**	**0.26**	**0.46**	**0.92 ***	**1.8**	**4.1**
	0	0	0	0	0	0
Cumulative mortality after 7 d [%]						
Cumulative number of molts after 7 d	3	2	1	3	3	7
Endpoints [mg iprodione/L] (mean measured)						
LC_{50} (7 d)	> 4.1					
NOEC (7 d)	≥ 4.1					

* One crayfish was observed to be missing from this test chamber at day 3, which was assumed to be the result of cannibalism.

III. CONCLUSION

In a flow-through, acute toxicity study with juvenile crayfish (*Procambarus simulans*), the LC_{50} (7 d) for iprodione was > 4.1 mg a.s./L based on mean measured concentrations. The NOEC was determined to be ≥ 4.1 mg iprodione/L (mean measured).

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to Daphnia magna

No further studies required; thus, this point is not addressed *via* new toxicity studies.

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

The following chronic toxicity study on the marine saltwater mysid *Americamysis bahia* (former name: *Mysidopsis bahia*) performed with the active substance iprodione is not required for registration in the EU. However, the study was conducted due to U.S. data requirements and it is provided for the sake of completeness. It has not been evaluated previously on EU level.

Report:	CA 8.2.5.2/1 Surprenant D.C., 1988a Chronic toxicity of Iprodione technical to mysid shrimp (Mysidopsis bahia) C022008
Guidelines:	EPA 72-4
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The chronic toxicity of iprodione to saltwater mysids (*Americamysis bahia*) was evaluated in a 28-day full-life cycle test under flow-through conditions. Mysids were exposed to a dilution water control, a solvent control and to nominal test item concentrations of 0.031, 0.063, 0.13, 0.25 and 0.50 mg iprodione/L (corresponding to mean measured concentrations of 0.015, 0.031, 0.063, 0.10 and 0.25 mg a.s./L). Survival, reproductive success and symptoms of toxicity were assessed throughout the study. Dry weight of the males and females was determined at test termination.

The biological results are based on mean measured concentrations. Survival of saltwater mysids was statistically significantly affected compared to the pooled control at the two highest test item concentrations of 0.10 and 0.25 mg iprodione/L. Due to statistically significant reduced survival at the two highest test item concentrations, reproduction and growth data for these treatment levels was not statistically compared to control data. Reproductive success showed statistically significant differences to the pooled control at the test item concentrations of 0.015, 0.031 and 0.063 mg iprodione/L. Evaluation of dry weight data (in the remaining three treatment levels) showed that the dry weights of the surviving adult male and female mysids were not statistically significantly different from the pooled control data.

In a flow-through chronic toxicity study with saltwater mysids (*Americamysis bahia*), the overall NOEC (28 d) for iprodione was determined to be < 0.015 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Iprodione (BAS 610 F; Reg. no.: 101 169), purity: 100%.

B. STUDY DESIGN

Test species: Saltwater mysid (*Mysidopsis bahia;* nowadays called: *Americamysis bahia*), juveniles, age: less than 24 hours old; source: in-house culture.

Test design: Flow-through system (28 d); 5 test item concentrations plus a control and a solvent control, 2 replicates for each test item concentration, the control and the solvent control; 30 mysids per glass aquaria (15 mysids per test chamber); mysids were maintained in test chambers until sexual maturity; at time of sexual maturity (day 15) male-female pairs were transferred into pairing chambers (10 pairing chambers per glass aquaria); remaining mysids (after isolation of male-female pairs) were pooled and placed in separate test chambers within glass aquaria; dead parental mysids and juveniles released during the test were removed; daily assessment of survival and symptoms of toxicity, reproduction (number of offspring produced by each female) from day 15 on; determination of dry weight at test termination.

Endpoints: NOEC based on survival, reproductive success and dry weight.

Test concentrations: Control (dilution water), solvent control (88µL/L acetone); 0.031, 0.063, 0.13, 0.25 and 0.50 mg iprodione/L (nominal), corresponding to mean measured concentrations of 0.015, 0.031, 0.063, 0.10 and 0.25 mg iprodione/L.

Test conditions: 19 L glass aquaria with two test chambers, test chambers: glass petri dishes (10 cm diameter, 2 cm depth) with a 15 cm high nylon screen collar (363 µm mesh size) attached, pairing chambers: cylindrical glass jars (5.1 cm diameter, 10 cm height) with two 1.9 cm holes covered with nylon screens (363 µm mesh size); dilution water: filtered natural seawater, flow rate: 0.5 L/per cycle/aquarium resulting in approximately 7 volume exchanges/aquarium/24 h; salinity: approx. 30 - 32 g/L; temperature: 23 - 26 °C; pH 7.6 - 7.8; oxygen content: 4.0 mg/L - 8.9 mg/L; photoperiod 16 h light : 8 h dark; light intensity: 15 - 110 foot candles; mysids were fed with live brine shrimps (*Artemia salina*) supplemented with a mixture of proteins and fatty acids at a minimum of twice daily.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with variable wavelength detection.

Statistics: Descriptive statistics; one-way ANOVA for comparison of the control data ($\alpha = 0.05$); Williams's test for determination of NOEC values ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at test initiation and after 7, 14, 23, and 28 days. Measured concentrations for iprodione were between 38.0% and 56.2% of nominal at test initiation. Measured concentrations after 7, 14, and 23 days ranged from 28.2% to 53.7%, from 35.4% to 61.4%, from 48.0% to 78.0% of nominal, respectively. At test termination, measured concentrations were between 48.0% and 68.0% 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 (one-way, single classification ANOVA; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. Survival of saltwater mysids was statistically significantly affected compared to the pooled control at the two highest test item concentrations of 0.10 and 0.25 mg iprodione/L (Williams test, $\alpha = 0.05$). Due to statistically significant reduced survival at the two highest test item concentrations, reproduction and growth data for these treatment levels was not statistically compared to control data. Reproductive success showed statistically significant differences to the pooled control data at the test item concentrations of 0.015, 0.031 and 0.063 mg iprodione/L. Evaluation of dry weight data (in the remaining three treatment levels) showed that the dry weights of the surviving adult male and female mysids were not statistically significantly different from the pooled control data. The results are summarized in Table 8.2.5.2-1.

Table 8.2.5.2-1: Chronic toxicity (28 d) of iprodione to saltwater mysids (*Americamysis bahia*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**Pooled control** +	**0.031**	**0.063**	**0.13**	**0.25**	**0.50**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**--**	**0.015**	**0.031**	**0.063**	**0.10**	**0.25**
Survival on day 28 [%]	82	87	84	87	70	80	65 *	42 *
Reproductive success [Offspring per female per reproductive day]	0.86 ± 0.35	0.75 ± 0.29	0.80 ± 0.32	0.47 ± 0.31 *	0.25 ± 0.25 *	0.34 ± 0.35 *	0.087 ± 0.15 #	0.047 ± 0.14 #
Dry weight on day 28, males [mg]	0.79 ± 0.14	0.72 ± 0.13	0.74 ± 0.16	0.75 ± 0.13	0.76 ± 0.14	0.71 ± 0.17	0.61 ± 0.11 #	0.66 ± 0.14 #
Dry weight on day 28, females [mg]	0.97 ± 0.18	0.89 ± 0.34	0.93 ± 0.18	0.87 ± 0.18	0.86 ± 0.15	0.82 ± 0.13	0.78 ± 0.16 #	0.77 ± 0.18 #
Endpoints [mg iprodione/L] (mean measured)								
NOEC $_{overall}$ (28 d)	< 0.015 (extrapolated value: 0.0030)							

+ Mean of control and solvent control data; control and solvent control data were not significantly different (one-way, single classification ANOVA; $\alpha = 0.05$).

* Statistically significant differences compared to the pooled control (Williams test for survival data $\alpha = 0.05$).

\# Due to statistically significant reduced survival at this test item concentration, reproduction and growth data for this treatment level was not statistically compared to control data.

III. CONCLUSION

In a flow-through chronic toxicity study with saltwater mysids (*Americamysis bahia*), the overall NOEC (28 d) for iprodione was determined to be < 0.015 mg a.s./L based on mean measured concentrations.

The following chronic toxicity study on the marine saltwater mysid *Americamysis bahia* (former name: *Mysidopsis bahia*) performed with the active substance iprodione is not required for registration in the EU. However, the study was conducted due to U.S. data requirements and it is provided for the sake of completeness. It has not been evaluated previously on EU level. This second chronic study on *A. bahia* replaces the former study (Surprenant, DocID C022008; see above) where no clear endpoint could be determined at the tested concentration levels.

Report:	CA 8.2.5.2/2 Surprenant D.C., 1988b Chronic toxicity of Iprodione technical to mysid shrimp (Mysidopsis bahia) C026001
Guidelines:	EPA 72-4
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The chronic toxicity of iprodione to saltwater mysids (*Americamysis bahia*) was evaluated in a 28-day full-life cycle test under flow-through conditions. Mysids were exposed to a dilution water control, a solvent control and to nominal test item concentrations of 0.0042, 0.0084, 0.017, 0.034 and 0.067 mg iprodione/L (corresponding to mean measured concentrations of 0.0035, 0.0075, 0.014, 0.025 and 0.055 mg a.s./L). Survival, reproductive success and symptoms of toxicity were assessed throughout the study. Dry weight of the males and females was determined at test termination.

The biological results are based on mean measured concentrations. Survival of saltwater mysids was statistically significantly affected compared to the pooled control at the highest test item concentration of 0.055 mg iprodione/L. Due to statistically significant reduced survival at the highest test item concentration, reproduction and growth data for this treatment level was not statistically compared to control data. Reproductive success at the test item concentrations of 0.014 and 0.025 mg a.s./L was statistically significantly different from the pooled control. Comparison of dry weight data for males and females did not establish statistically significant effects, compared to pooled control data.

In a flow-through chronic toxicity study with saltwater mysids (*Americamysis bahia*), the overall NOEC (28 d) for iprodione was determined to be 0.0075 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Iprodione (BAS 610 F; Reg. no.: 101 169), purity: 100%.

B. STUDY DESIGN

Test species: Saltwater mysid (*Mysidopsis bahia;* nowadays called: *Americamysis bahia*), juveniles, age: less than 24 hours old; source: in-house culture.

Test design: Flow-through system (28 d); 5 test item concentrations plus a control and a solvent control, 2 replicates for each test item concentration, the control and the solvent control; 30 mysids per glass aquaria (15 mysids per test chamber); mysids were maintained in test chambers until sexual maturity; at time of sexual maturity (day 16) male-female pairs were transferred into pairing chambers (10 pairing chambers per glass aquaria); remaining mysids (after isolation of male-female pairs) were pooled and placed in separate test chambers within glass aquaria; dead parental mysids and juveniles released during the test were removed; daily assessment of survival and symptoms of toxicity, reproduction (number of offspring produced by each female) from day 16 on; determination of dry weight at test termination.

Endpoints: NOEC based on survival, reproductive success and dry weight.

Test concentrations: Control (dilution water), solvent control (13 µL/L acetone); 0.0042, 0.0084, 0.017, 0.034 and 0.067 mg iprodione/L (nominal), corresponding to mean measured concentrations of 0.0035, 0.0075, 0.014, 0.025 and 0.055 mg a.s./L.

Test conditions: 19 L glass aquaria with two test chambers, test volume: 4 - 7 L, test chambers: glass petri dishes (10 cm diameter, 2 cm depth) with a 15 cm high nylon screen collar (363 µm mesh size) attached, pairing chambers: cylindrical glass jars (5.1 cm diameter, 10 cm height) with two 1.9 cm holes covered with nylon screens (363 µm mesh size); dilution water: filtered natural seawater; flow rate: 0.5 L/per cycle/aquarium resulting in approximately 7 volume exchanges/aquarium/24 h; temperature: 25 ± 1 °C; pH 7.7 - 8.1; oxygen content: 4.5 mg/L - 7.6 mg/L; photoperiod 16 h light : 8 h dark; light intensity: 30 - 110 foot candles; mysids were fed with live brine shrimps (Artemia salina) supplemented with a mixture of proteins and fatty acids at a minimum of twice daily.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with variable wavelength detection.

Statistics: Descriptive statistics; one-way ANOVA for comparison of the control data ($p < 0.05$); Williams's test, Dunnett's test or Kruskal-Wallis test for determination of NOEC values ($p < 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at test initiation, on days 7, 14, 21, 24 and at test termination. Measured concentrations for iprodione were between 46.4% and 79.2% of nominal at test initiation. Measured concentrations after 7, 14, 21 and 24 days ranged from 61.8% to 94.0%, from 41.2% to 61.9%, from 111.8% to 121.6% and from 88.2% to 117.9% of nominal, respectively. At test termination, measured concentrations were between 64.4% and 78.6% of nominal. The following biological results are based on mean measured concentrations.

Biological results: Statistical comparison between the performance of the control and solvent control organisms established that the solvent control organisms released significantly more offspring than the control mysids (one way ANOVA, $p <\_ 0.05$). However, since exposure to the solvent control did not have a detrimental effect, all statistical analysis for each toxic endpoint were performed by comparing the treatment data against the pooled control data to determine significant effects. Survival of saltwater mysids was statistically significantly affected compared to the pooled control at the highest test item concentration of 0.055 mg iprodione/L (Williams's test, Dunnett's test or Kruskal-Wallis test; $p < 0.05$). Due to statistically significant reduced survival at the highest test item concentration, reproduction and growth data for this treatment level was not statistically compared to control data. Reproductive success at the test item concentrations of 0.014 and 0.025 mg a.s./L was statistically significantly different from the pooled control. Comparison of dry weight data for males and females did not establish statistically significant effects, compared to pooled control data. The results are summarized in Table 8.2.5-2.

Table 8.2.5-2: Chronic toxicity (28 d) of iprodione to saltwater mysids (*Americamysis bahia*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**Pooled control**	**0.0042**	**0.0084**	**0.017**	**0.034**	**0.067**
Concentration [mg a.s./L] (mean measured)	--	--	--	**0.0035**	**0.0075**	**0.014**	**0.025**	**0.055**
Survival on day 28 [%]	92	89	90	89	88	75	83	57 *
Reproductive success [offspring per female per reproductive day]	0.52 ± 0.31	0.67 ± 0.25	0.59 ± 0.29	0.60 ± 0.27	0.57 ± 0.28	0.37 ± 0.24 *	0.27 ± 0.20 *	0.15 ± 0.18 #
Dry weight on day 28, males [mg]	0.67 ± 0.09	0.64 ± 0.16	0.66 ± 0.13	0.58 ± 0.10	0.69 ± 0.13	0.65 ± 0.11	0.64 ± 0.10	0.61 ± 0.11 #
Dry weight on day 28, females [mg]	0.81 ± 0.15	0.88 ± 0.24	0.84 ± 0.20	0.60 ± 0.10	0.87 ± 0.17	0.70 ± 0.21	0.76 ± 0.16	0.69 ± 0.11 #
Endpoints [mg iprodione/L] (mean measured)								
NOEC $_{overall}$ (28 d)	0.0075							

* Statistically significant differences compared to the pooled control (Williams's test, Dunnett's test or Kruskal-Wallis test; $p < 0.05$).

\# Since survival at this test item concentration was adversely affected, reproduction and growth data for this treatment level was not statistically compared to control data.

III. CONCLUSION

In a flow-through chronic toxicity study with saltwater mysids (*Americamysis bahia*), the overall NOEC (28 d) for iprodione was determined to be 0.0075 mg a.s./L based on mean measured concentrations.

The following chronic toxicity study on the Cladoceran, *Ceriodaphnia dubia* performed with the active substance iprodione is provided in support of the aquatic risk assessment and has not been evaluated previously on EU level. The study was performed to provide additional information on the chronic toxicity of iprodione to aquatic invertebrates.

Report: CA 8.2.5.2/3
Hicks S., 2014a
BAS 610F: Chronic toxicity test with Ceriodaphnia dubia conducted under flow through conditions
2013/7002669
Guidelines:
GLP: yes

Executive Summary

The chronic toxicity of iprodione to the cladoceran, *Ceriodaphnia dubia,* was investigated in an 8-day test under flow-through conditions. Test animals were exposed to a dilution water control and to nominal test item concentrations of 0.025, 0.050, 0.10, 0.20, 0.40, 0.80 and 1.6 mg iprodione/L (corresponding to geometric mean measured concentrations of 0.0196, 0.0297, 0.0595, 0.122, 0.237, 0.471 and 1.30 mg a.s./L). Immobilization, abnormalities and reproduction were recorded daily over the exposure period of 8 days.

The biological results are based on geometric mean measured concentrations. After 8 days of exposure, no mortality/immobility of *C. dubia* was observed in the control and survival rates in the test item treatments ranged from 50% to 100%. The survival rates of 50% in the test item treatments of 0.0595 and 0.471 mg a.s./L were statistically significantly different from the control. However, the reduction in survival found in the 0.0595 mg a.s./L treatment was not indicative of a dose-response trend because survival in the next highest treatment was 100%, and therefore was considered not to be biologically significant. The total numbers of live young produced by surviving first generation daphnid after an 8-day exposure were 167 in the control and between 27 and 128 in the test item treatments. The mean number of live young produced per surviving adult during the course of the test was 17 in the control and between 5 and 13 in the test item treatments. Statistically significant reductions in the number of live young per surviving adult compared to the control was observed at geometric mean measured concentrations of 0.237, 0.471, and 1.30 mg a.s./L.

In a chronic flow-through toxicity study with *Ceriodaphnia dubia*, the overall NOEC (8 d) for iprodione was determined to be 0.122 mg a.s./L, based on geometric mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Iprodione (BAS 610 F; Reg. no.: 101 169), batch no. COD-001260; purity: 97.8%.

B. STUDY DESIGN

Test species: Cladoceran (*Ceriodaphnia dubia)*, neonates, less than 24 hours old; source: in-house culture.

Test design: Flow-through system (8 d); 7 test item concentrations plus a control, ten replicates per treatment with one parent daphnid in each; daily assessment of surviving adults (*i.e.* immobility), occurrence of abnormalities and reproductive performance over the 8 day exposure period.

Endpoints: NOEC based on immobility and reproductive success.

Test concentrations: Control (dilution water); 0.025, 0.050, 0.10, 0.20, 0.40, 0.80 and 1.6 mg iprodione/L (nominal), corresponding to geometric mean measured concentrations of < MQL (control, minimum quantification level), 0.0196, 0.0297, 0.0595, 0.122, 0.237, 0.471 and 1.30 mg iprodione/L.

Test conditions: 100 mL glass beakers, test volume 50 mL, dilution water synthetic freshwater (aerated, deionized well water prepared according to EPA 821-R-02-013); flow rate: 0.5 L/per cycle/test chamber resulting in approximately 5 volume exchanges/test chamber/24 h (approx. 0.208 cycles/hour); temperature: 24.1 °C - 25.9 °C; pH 7.51 - 8.19; oxygen content: 7.5 mg/L - 9.2 mg/L (93% to 116% of saturation); total hardness: 86 - 88 mg $CaCO_3$/L, conductivity: 338 µS/cm - 343 µS/cm; light intensity: 282 lux - 350 lux; photoperiod 16 hours light : 8 hours dark; feeding with algal suspension and a suspension of an artificial invertebrate diet; the daphnids received this diet once on the days the study started and ended, and three times between these days; no aeration.

Analytics: Analytical verification of test item concentrations was conducted using LC-MS/MS-method.

Statistics: Descriptive statistics; determination of NOEC values using one-tailed Dunnett's test ($\alpha = 0.05$) for determination of NOEC values based on mortality/immobility and reproduction data.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in all treatments at day 0, 2, 4 and 8. Mean measured concentrations for iprodione were between 51% and 80% of the nominal concentrations at test initiation and between 71% and 102% of nominal at test termination. The geometric mean measured concentrations of the test substance treatment solutions were 0.0196, 0.0297, 0.0595, 0.122, 0.237, 0.471, and 1.30 mg a.s./L for the 0.025, 0.050, 0.10, 0.20, 0.40, 0.80, and 1.6 mg a.s./L nominal test substance treatments. The geometric mean measured concentrations ranged from 59% to 81% of the nominal concentrations. No residues of iprodione were detected in the control solutions above the MQL of 0.00500 mg a.s./L. The recoveries of the 0.0199 mg a.s./L QC fortification samples ranged from 84% to 117% of the nominal concentrations, with the exception of the day 5 sample which was 59% of the nominal concentration. The recoveries of the 2.07 mg a.s./L QC fortification samples ranged from 78% to 111% of the nominal concentrations, with the exception of the day 5 sample which was 64% of the nominal concentration. The biological results were based on the geometric mean measured concentrations.

Biological results: After 8 days of exposure, no mortality/immobility of *C. dubia* was observed in the control and survival rates in the test item treatments ranged from 50% to 100%. The survival rates of 50% in the test item treatments of 0.0595 and 0.471 mg a.s./L (geometric mean measured) were statistically significantly different from the control (Dunnett's test; $p = 0.05$). However, the reduction in survival found in the 0.0595 mg a.s./L treatment was not indicative of a dose-response trend because survival in the next highest treatment was 100%, and therefore was considered not to be biologically significant.
The total numbers of live young produced by surviving first generation daphnid after an 8-day exposure were 167 in the control and between 27 and 128 in the test item treatments. The mean number of live young produced per surviving adult during the course of the test was 17 in the control and between 5 and 13 in the test item treatments. Statistically significant reductions in the number of live young per surviving adult compared to the control was observed at geometric mean measured concentrations of 0.237, 0.471, and 1.30 mg a.s./L (Dunnett's test, $\alpha = 0.05$).

The results are summarized in Table 8.2.5.2-1.

Table 8.2.5.2-3: Chronic toxicity (8 d) of iprodione to water flea (*Ceriodaphnia dubia)*

Concentration [mg a.s./L] (nominal)	**Control**	**0.025**	**0.050**	**0.10**	**0.20**	**0.40**	**0.80**	**1.6**
Concentration [mg a.s./L] (geometric mean measured)	**< MLQ**	**0.0196**	**0.0297**	**0.0595**	**0.122**	**0.237**	**0.471**	**1.30**
Survival on day 8 [%]	100	70	90	50 * [1)]	100	60	50 *	60
Reproductive success [total number of live young]	167	91	99	59	128	55	45	27
Reproductive success [mean number of live young per surviving adult]	17	13	11	12	13	9 *	9 *	5 *
Time to first brood release	5	5	6	6	5	6	6	7
Endpoints [mg iprodione/L] (geometric mean measured)								
NOEC $_{survival}$ (8 d)	0.237							
NOEC $_{reproduction}$ (8 d)	0.122							

MQL = minimum quantification level

* Statistically significant reduction (Dunnett's test; $p = 0.05$) as compared to the control.

[1)] The reduction found in this treatment was not indicative of a dose-response trend because survival in the next highest treatment was 100% and therefore was considered not to be biologically significant.

III. CONCLUSION

In a chronic flow-through toxicity study with *Ceriodaphnia dubia*, the overall NOEC (8 d) for iprodione was determined to be 0.122 mg a.s./L, based on geometric mean measured concentrations.

A chronic toxicity test on the freshwater amphipod, *Hyalella azteca*, was initiated to assess the effects of iprodione on survival, growth, and reproduction of the amphipod. The rationale for this was to refine the chronic risk assessment for aquatic invertebrates. However, due to unexpected, but significant problems of this 42 day study, which were not to be mastered within any reasonable time frame around the submission of this reregistration dossier, is was decided (after consultation with the RMS) to abandon this study.

Report: CA 8.2.5.2/4
Hicks S., 2014b
Study terminated - BAS 610F: A spiked water chronic toxicity test with Hyallela azteca conducted under flow-through condition
2013/7002670
Guidelines:
GLP: yes

Executive Summary

The study was terminated.

The major problems were the reproduction performance and high mortality of the control animals and the non-availability of a suitable substrate for this modified non-standard test (i.e. spiked water instead of sediment). The hydrolytical instability of the compound further impeded the test. More details on the encountered difficulties and the non-conclusive data from the 12 day range finding study are given in the interim report.

The collected did not allow to draw any conclusions on the chronic toxicity of the test substance. The study is not considered in aquatic risk assessment of iprodione.

CA 8.2.5.3 Development and emergence in Chironomus riparius

The following chronic toxicity study on *Chironomus riparius* performed with the metabolite RP 30228 is provided in support of the aquatic risk assessment and has not been evaluated previously. The study is required, due to new data requirements.

Report:	CA 8.2.5.3/1 Kuhl R.,Wydra V., 2013a Effects of Reg.No. 5079647 (metabolite of BAS 610 F, Iprodione, RP30228) on the development of sediment dwelling larvae of Chironomus riparius in a sediment-water system - Exposed via spiked sediment 2013/1000181
Guidelines:	OECD 218: Sediment-water chironomid toxicity test using spiked sediment (April 2004)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

In a 28-day static spiked sediment study, non-biting midge larvae (*Chironomus riparius*) were exposed to RP 30228 (metabolite of iprodione). Nominal test concentrations were 6.25, 12.5, 25.0, 50.0 and 100.0 mg RP 30228/kg dry sediment (corresponding to initially measured concentrations of 5.84, 9.80, 20.88, 49.66 and 95.34 mg RP 30228/kg dry sediment). Additionally, a solvent (acetone) control and a dilution water control were set up. All test item concentrations and the water control had 4 replicates, whereas 6 replicates were tested for the solvent control. 20 larvae were added to each test vessel,

The biological results are based on initially measured concentrations. First emerged midges were observed 14 days after insertion of larvae. In the control and all test item treatments 16 to 30 midges emerged. At test end, mean emergence rates of 95.0% and 95.8% and mean development rates of 0.060 and 0.064 were observed in the control and the solvent control, respectively. No statistically significant differences were found for the emergence and development rates at any test item concentration when compared to the pooled control.

In a 28-day static sediment test with *Chironomus riparius* the NOEC values of RP 30228 (metabolite of iprodione) for emergence rate and development rate were determined to be both ≥ 95.34 mg/kg dry sediment based on initially measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: RP 30228 (Reg. No. 5 079 647, metabolite of iprodione), batch no. DP646B, purity: 99.8%.

B. STUDY DESIGN

Test species: Non-biting midge (*Chironomus riparius*), first instar larvae, 3 days old at test initiation; source: in-house culture.

Test design: Static system (28 days); 5 test concentrations plus a solvent (acetone) control and a water control, 4 replicates per test item concentration and for the water control, 6 replicates for the solvent control; 20 larvae per test vessel; assessment of emergence rate and development rate.

Endpoints: NOEC and EC_{50} (regarding emergence rate and development rate).

Test concentrations: Water control, solvent control, 6.25, 12.5, 25.0, 50.0 and 100.0 mg RP 30228/kg dry sediment (nominal), corresponding to initially measured concentrations of 5.84, 9.80, 20.88, 49.66 and 95.34 mg RP 30228/kg dry sediment.

Test conditions: 600 mL glass vessels with 100 g (± 1 g) spiked wet artificial sediment (according to OECD 218), 280 mL M4 water (Elendt medium) corresponding to a water layer of 6 cm; pH 8.1 - 8.6; oxygen concentration: 74% - 108%; total hardness: 294 - 320 mg $CaCO_3$/L; conductivity: 613 µS/cm; ammonia: 0.2 mg/L at test initiation (DAI 0) and 5.0 mg/L at test termination (DAI 28); water temperature: 19 °C - 21 °C; light intensity: 590 lux - 780 lux; photoperiod: 16 h light : 8 h dark; continuous gentle aeration; food: 0.5 mg TetraMin per larva per day from DAI 0 to DAI 09 and 0.5 - 1.0 mg TetraMin per larva per day from DAI 10 to DAI 20.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection and an LC-method using MS/MS detection.

Statistics: Descriptive statistics, Student-t-test ($p < 0.05$) for comparison of the emergence rates and development rates the effects in the control groups; Williams t-test procedure for comparison of the emergence rate and development rate in the treatment groups to the pooled control ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations in the sediment, the overlaying water and the pore water was conducted in each concentration at the beginning (DAI 0) and the end of the test (DAI 28). Recoveries in the sediment were in a range between 78% - 99% of the nominal concentrations at test initiation. At test termination the detected concentrations ranged from 71% - 88% of the nominal values. Overlaying water concentrations ranged from 0.016 to 0.062 mg RP 30228/L on DAI 0 and from 0.017 to 0.066 mg RP 30228/L on DAI 28. The pore water concentrations measured on DAI 0 were between 0.037 and 0.479 mg RP 30228/L and between 0.030 and 0.222 mg RP 30228/L on DAI 28. The following biological results are based on the initially measured sediment concentrations.

Biological results: First emerged midges were observed on 14 days after insertion of larvae. In the control and all test item treatments 16 to 30 midges emerged. At test end, mean emergence rates of 95.0% and 95.8% and mean development rates of 0.060 and 0.064 were observed in the control and the solvent control, respectively. No statistically significant difference was observed between the controls (Student-t-test, $p < 0.05$). Hence, the controls were pooled and used as the reference in all evaluations. No statistically significant differences were found for the emergence and development rates (Williams t-test, $\alpha = 0.05$) at any test item concentration when compared to the pooled control. The results are summarized in Table 8.2.5.3-1.

Table 8.2.5.3-1: Effects of RP 30228 (metabolite of iprodione) on emergence and development of *Chironomus riparius* (28 d)

Concentration [mg RP 30228/kg dry sediment] (nominal)	**Solvent control**	**water control**	**6.25**	**12.5**	**25.0**	**50.0**	**100.0**
Concentration [mg RP 30228/kg dry sediment] (initially measured)	--	--	**5.84**	**9.80**	**20.88**	**49.66**	**95.34**
Mean emergence rate (28 d) [% emerged]	95.8 ± 2.0	95.0 ± 7.1	97.5 ± 2.9	98.8 ± 2.5	95.0 ± 4.1	93.8 ± 9.5	97.5 ± 2.9
[% of pooled control]	--	--	102	103	100	98	102
Mean development rate (28 d)	0.064	0.060	0.063	0.061	0.060	0.059	0.060
[% of pooled control]	--	--	102	98	96	96	96
	Endpoints [mg RP 30228/kg dry sediment] (initially measured)						
$EC_{50\ emergence\ rate\ /\ development\ rate}$	> 95.34						
$NOEC_{emergence\ rate\ /\ development\ rate}$	≥ 95.34						

III. CONCLUSION

In a 28-day static sediment test with *Chironomus riparius* the NOEC values of RP 30228 (metabolite of iprodione) for emergence rate and development rate were determined to be both ≥ 95.34 mg/kg dry sediment based on initially measured concentrations.

CA 8.2.5.4 Sediment dwelling organisms

No further studies required; thus, this point is 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

No further studies required; thus, this point is not addressed *via* new toxicity studies.

CA 8.2.6.2 Effects on growth of an additional algal species

The following alga toxicity studies on the freshwater diatom *Navicula pelliculosa,* blue-green alga *Anabaena flos-aquae and marine diatom* Skeletonema costatum performed with the active substance iprodione are not required for registration in the EU. The studies were conducted due to U.S. data requirements and have not been evaluated previously on EU level.
Following the recommendations and validity criteria defined in the current OECD guideline 201 for alga testing (OECD, 2011) the 120 h studies on the algae *N. pelliculosa, A. flos-aquae* and *S. costatum* are considered to be not valid. In all three studies, at least one validity criterion is not met:

- the mean coefficients of variation (CV) for section by section specific growth rate in both the control and the solvent control were significantly > 35% after 0 - 72 hours and 0 - 120 hours in all three studies;
- the CV of average specific growth rate in both the control and the solvent control was > 7% after 0 - 72 hours of exposure in the studies on *N. pelliculosa* and *A. flos-aquae;*
- the biomass increase over the first 96 h in the controls were < 16 fold in the study on *N. pelliculosa* (*i.e.* max. 5-fold after 96 h in the solvent control*)*; thus, no sufficient growth over the first 96 h of the test is demonstrated (*i.e.* in some replicates of the control cell densities were zero over the first 96 hours);

Furthermore, a very high variability among the cell density data of the single replicates in the controls and the test item treatments was shown in the studies with *N. pelliculosa* and *A. flos-aquae* over the first 96 hours. (*i.e.* coefficients of variation partly > 100% even in the controls).

Nevertheless, summaries of the studies are provided for the sake of completeness.

Report:	CA 8.2.6.2/1 Giddings J.M., 1990a Iprodione technical - Toxicity to the freshwater diatom Navicula pelliculosa C021737
Guidelines:	EPA 123-2
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 120-hour static toxicity laboratory study, the effect of iprodione on of the growth of the freshwater diatom *Navicula pelliculosa* was investigated. The following nominal concentrations were applied: 0.016, 0.033, 0.065, 0.13, 0.25 and 0.50 mg iprodione/L (corresponding to mean measured concentrations of 0.013, 0.020, 0.047, 0.094, 0.19 and 0.26 mg a.s./L). Additionally, a solvent (acetone) control 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. In cultures exposed to test item concentrations of 0.094 mg/L and higher, cell walls were observed to be thin. Cells were observed to be pale in cultures exposed to test item concentrations of 0.19 mg/L and higher. In the 0.26 mg/L treatment, fragmented cells were observed.

In a 120 hour algae toxicity test with *Navicula pelliculosa,* the E_bC_{50} (120 h) for iprodione was determined to be 0.048 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Iprodione (BAS 610 F; Reg. no.: 101 169), lot no. 8906201; purity: 96.2%

B. STUDY DESIGN

Test species: Freshwater diatom, *Navicula pelliculosa*; stock obtained from the "Carolina Biological Supply Company", Burlington, North Carolina, USA.

Test design: Static system; test duration 120 hours; 6 test concentrations with 3 replicates for each test item concentration, the control and the solvent control; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to biomass after exposure over 120 hours.

Test concentrations: Control, solvent control (0.10 mL acetone/L), 0.016, 0.033, 0.065, 0.13, 0.25 and 0.50 mg iprodione/L (nominal), corresponding to mean measured concentrations of 0.013, 0.020, 0.047, 0.094, 0.19 and 0.26 mg iprodione/L.

Test conditions: 125 mL flasks; test volume 50 mL; MBL medium excluding EDTA; pH 7.4 at test initiation, pH 7.5 at test termination; temperature: 22 °C - 27 °C; initial cell densities 0.3 x 10^4 cells/mL; continuous light at 4000 - 5000 lux; constant shaking.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with variable wavelength detection.

Statistics: Descriptive statistics; t-test (p = 0.01) for comparison of cell densities in the control and solvent control; linear regression analysis for determination of EC_x values, Bonferroni test for determination of the NOEC value.

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 iprodione ranged from 75% to 116% of nominal concentrations at test initiation and from 28% to 42% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: In cultures exposed to test item concentrations of 0.094 mg/L and higher, cell walls were observed to be thin. Cells were observed to be pale in cultures exposed to test item concentrations of 0.19 mg/L and higher. In the 0.26 mg/L treatment, fragmented cells were observed. Statistically significant differences compared to the pooled control were observed at the five highest test item concentrations (Bonferroni`s test). The effects on algal biomass are summarized in Table 8.2.6.2-1.

Table 8.2.6.2-1: Effect of iprodione on biomass development of the freshwater diatom *Navicula pelliculosa*

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.016**	**0.033**	**0.065**	**0.13**	**0.25**	**0.50**
Concentration [mg a.s./L] (mean measured)	--	--	**0.013**	**0.020**	**0.047**	**0.094**	**0.19**	**0.26**
Mean cell density (120 h) [x 10^4 cells/mL]	16.00	15.00	17.33	6.08 *	6.83 *	5.00 *	3.75 *	1.75 *
Inhibition in 120 h (biomass) [1), 2)] [% of pooled control] [3)]	--	--	-11.8	60.8	55.9	67.7	75.8	88.7
	Endpoints [mg iprodione/L] (mean measured)							
E_bC_{10} (120 h)	0.0090 (95% confidence limits: 0.00068 - 0.060)							
E_bC_{50} (120 h)	0.048 (95% confidence limits: 0.0059 - 0.36)							
NOEC (120 h)	0.013							

* Statistically significant differences compared to the pooled control (Bonferroni`s test).
1) Negative values indicate stimulated growth compared to the control.
2) Inhibition values were calculated from mean cell density values.
3) No significant difference between control and solvent control cultures (t-test, $\alpha = 0.01$), therefore data from the control and solvent control was pooled for EC_{50} and NOEC calculation.

III. CONCLUSION

In a 120 hour algae toxicity test with *Navicula pelliculosa,* the E_bC_{50} (120 h) for iprodione was determined to be 0.048 mg a.s./L based on mean measured concentrations.

Report:	CA 8.2.6.2/2 Giddings J.M., 1990b Iprodione technical - Toxicity to the freshwater bluegreen alga Anabaena flos-aquae C021725
Guidelines:	EPA 123-2
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 120-hour static toxicity laboratory study, the effect of iprodione on growth of the blue-green alga *Anabaena flos-aquae* was investigated. The following nominal concentrations were applied: 0.03, 0.06, 0.13, 0.26, 0.50, 1.0 and 2.0 mg iprodione/L (corresponding to initially measured concentrations of 0.029, 0.069, 0.13, 0.25, 0.45, 0.82 and 1.3 mg a.s./L). Additionally, a solvent (acetone) control and a dilution water control were set up. Assessment of biomass development was conducted 24 h, 48 h, 72 h, 96 h and 120 h after test initiation.

The biological results are based on initially measured concentrations. After 120 hours of exposure, no morphological effects on algae were observed in the control group and at any of the test item concentrations tested. Furthermore, no statistically significant differences in alga growth compared to the control were observed at any test item concentration.

In a 120-hour algae toxicity test with *Anabaena flos-aquae*, the E_bC_{50} of iprodione was determined to be > 1.3 mg/L based on initially measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Iprodione (BAS 610 F, Reg. no. 101 169, lot no. 8906201): purity 96.2%.

B. STUDY DESIGN

Test species: Freshwater blue-green alga, *Anabaena flos-aquae*; in-house culture; stock originally obtained from "Carolina Biological Supply Company", Burlington, North Carolina, USA.

Test design: Static system (120 hours); 7 test concentrations with 3 replicates for each test item concentration, the control and the solvent control; daily assessment of growth.

Endpoints: EC_{50} with respect to biomass development after exposure over 120 hours.

Test concentrations: Control, solvent control (0.10 mL acetone/L), 0.03, 0.06, 0.13, 0.26, 0.50, 1.0 and 2.0 mg iprodione/L (nominal), corresponding to initially measured concentrations of 0.029, 0.069, 0.13, 0.25, 0.45, 0.82 and 1.3 mg iprodione/L.

Test conditions: 125 mL flasks plugged with stainless steel caps; test volume: 50 mL; AAP nutrient medium excluding EDTA; pH 7.4 - 7.6 at test initiation and pH 7.5 - 8.3 at test termination; temperature: 24 °C - 26 °C; initial cell densities: 0.3×10^4 cells/mL; continuous light at 900 - 2000 lux, continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with variable wavelength detection.

Statistics: Descriptive statistics, t-test ($\alpha = 0.05$) for comparison of cell densities in the control and solvent control; Bonferroni's test for determination of the NOEC value ($\alpha = 0.05$).

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 iprodione ranged from 64% to 108% of nominal concentrations at test initiation and from 21% to 34% of nominal at test termination. The following biological results are based on initially measured concentrations.

Biological results: After 120 hours of exposure, no morphological effects on algae were observed in the control group and at any of the test item concentrations tested. Furthermore, no statistically significant differences in alga growth compared to the control were observed at any test item concentration (Bonferroni's test, p = 0.05). The effects on algal biomass are summarized in Table 8.2.6.2-2.

Table 8.2.6.2-2: Effect of iprodione on the biomass of the blue-green alga *Anabaena flos-aquae*

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.03**	**0.06**	**0.13**	**0.26**	**0.50**	**1.0**	**2.0**
Concentration [mg a.s./L] (initially measured)	--	--	**0.029**	**0.069**	**0.13**	**0.25**	**0.45**	**0.82**	**1.3**
Mean cell density (120 h) [x 10^4 cells/mL]	24.33	34.42	36.75	24.33	32.33	34.58	35.50	36.08	38.92
Inhibition in 120 h (biomass) [1), 2)] [% of pooled control] [3)]	--	--	-25.1	17.2	-10.0	-17.7	-20.8	-22.8	-32.5
Endpoints [mg iprodione/L] (initially measured)									
E_bC_{50} (120 h)	> 1.3								

1) Negative values indicate stimulated growth compared to the control.
2) Inhibition values were calculated from mean cell density values.
3) No significant difference between control and solvent control cultures (t-test, α = 0.05), therefore data from the control and solvent control was pooled for EC_{50} calculation.

III. CONCLUSION

In a 120-hour algae toxicity test with *Anabaena flos-aquae*, the E_bC_{50} of iprodione was determined to be > 1.3 mg/L based on initially measured concentrations.

Report:	CA 8.2.6.2/3 Giddings J.M., 1990c Iprodione technical - Toxicity to the marine diatom Skeletonema costatum C021736
Guidelines:	EPA 123-2
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 120-h static toxicity laboratory study, the effect of iprodione on growth of the marine diatom *Skeletonema costatum* was investigated. The following nominal concentrations were applied: 0.030, 0.064, 0.13, 0.26, 0.50, 1.0 and 2.0 mg iprodione/L (corresponding to initially measured concentrations of 0.029, 0.070, 0.12, 0.23, 0.45, 0.68 and 1.1 mg a.s./L). Additionally, a solvent (acetone) control 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 initially measured concentrations. After 120 hours of exposure, statistically significant effect on biomass development in compared to the pooled control were observed at the test item concentrations of 0.12 mg a.s./L and all higher concentrations.

In a 120-h algae test with *Skeletonema costatum*, the E_bC_{50} of iprodione was determined to be 0.59 mg/L based on initially measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Iprodione (BAS 610 F; Reg. no.: 101 169), lot no. 8906201; purity 96.2%.

B. STUDY DESIGN

Test species: Marine diatom, *Skeletonema costatum*, in-house culture; stock originally obtained from the "Bigelow Laboratory", Boothbay Harbor, Maine, USA.

Test design: Static system (120 hours); 7 test concentrations with 3 replicates for each test item concentration, the control and the solvent control; daily assessment of growth.

Endpoints: EC_{50} and EC_{90} with respect to biomass development after exposure over 120 hours.

Test concentrations: Control, solvent control (0.10 mL acetone/L), 0.030, 0.064, 0.13, 0.26, 0.50, 1.0 and 2.0 mg iprodione/L (nominal), corresponding to initially measured concentrations of 0.029, 0.070, 0.12, 0.23, 0.45, 0.68 and 1.1 mg iprodione/L.

Test conditions: 125 mL flasks; test volume: 50 mL; Marine Algal Medium excluding EDTA; pH 7.7 - 8.0 at test initiation and pH 8.1 - 9.1 at test termination; temperature: 19 °C - 22 °C; initial cell densities: 1 x 10^4 cells/mL; photoperiod: 16 hours light : 8 hours dark, light intensity: 3500 - 5000 lux, continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC method with variable wavelength detection.

Statistics: Descriptive statistics, t-test ($\alpha = 0.05$) for comparison of cell densities in the control and solvent control; linear regression analysis for determination of EC_x values, Bonferroni's test or Kruskal-Wallis test for determination of the NOEC value.

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. At test initiation, measured concentrations of iprodione ranged from 55% to 109% of nominal concentrations. At test termination, iprodione was only detected at the highest test item concentration, in which the measured concentration was 9% of nominal. Possible causes of the loss of iprodione from the test solutions include uptake or sorption by algae, removal by photolysis or hydrolysis and biological degradation. The following biological results are based on initially measured concentrations.

Biological results: After 120 hours of exposure, statistically significant effect on biomass development in compared to the pooled control were observed at the test item concentrations of 0.12 mg a.s./L and all higher concentrations (Bonferroni`s test). The effects on algal biomass are summarized in Table 8.2.6.2-3.

Table 8.2.6.2-3: Effect of iprodione on the biomass of the marine diatom *Skeletonema costatum*

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.030**	**0.064**	**0.13**	**0.26**	**0.50**	**1.0**	**2.0**
Concentration [mg a.s./L] (initially measured)	--	--	**0.029**	**0.070**	**0.12**	**0.23**	**0.45**	**0.68**	**1.1**
Mean cell density (120 h) [x 10^4 cells/mL]	169.56	202.61	180.44	148.58	168.39	119.42 *	138.00 *	107.08 *	0.00 *
Inhibition in 120 h (biomass) [1] [% of pooled control] [3]	--	--	3.0	20.2	9.5	35.8	25.8	42.5	100.0
	Endpoints [mg iprodione/L] (initially measured)								
E_bC_{50} (120 h) *	0.59 (95% confidence limits: 0.17 - 1.0)								
NOEC (120 h) *	0.12								

* Statistically significant differences compared to the pooled control (Bonferroni`s test).
1) Inhibition values were calculated from mean cell density values.
2) No significant difference between control and solvent control cultures (t-test, $\alpha = 0.05$), therefore data from the control and solvent control was pooled for EC and NOEC calculation.

III. CONCLUSION

In a 120-h algae test with *Skeletonema costatum*, the E_bC_{50} of iprodione was determined to be 0.59 mg/L based on initially measured concentrations.

The following alga toxicity study on *Pseudokirchneriella subcapitata* performed with the metabolite RP 36221 is provided in support of the aquatic risk assessment and has not been evaluated previously. The study is required, due to new data requirements.

Report:	CA 8.2.6.2/4 Swierkot A., 2013d Reg. No. 5079618 (metabolite of BAS 610 F, Iprodione, RP036221 - Pseudokirchneriella subcapitata SAG 61.81 - Growth inhibition test 2013/1191246
Guidelines:	OECD 201 (2006)
GLP:	yes (certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Executive Summary

In a 72-hour static toxicity laboratory study, the effect of RP 36221 (metabolite of iprodione) on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following geometric mean measured concentrations were applied: 0 (control), 0.038, 0.077, 0.166, 0.366 and 0.709 mg RP036221/L. Assessment of growth was conducted 24 h, 48 h and 72 h after test initiation.

The biological results are based on geometric mean measured concentrations. No morphological effects on algae were observed in the control and at any of the test item concentrations tested. Statistically significant effects on algal growth rate and yield compared to the control were observed in all test item treatments after 72 hours of exposure.

In a 72-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} of RP 36221 (metabolite of iprodione) was determined to be 0.567 mg/L and the E_yC_{50} was 0.154 mg/L based on geometric mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: RP 36221 (Reg. No. 5 079 618, metabolite of iprodione), batch no. L80-172, purity: 98.4 ± 1%.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz); specification: SAG 61.81; stock obtained from "Sammlung von Algenkulturen", Göttingen, Germany.

Test design: Static system (72 hours); 5 test item concentrations with 3 replicates for each plus a control with 6 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours, NOEC.

Test concentrations: Control, 0.038, 0.077, 0.166, 0.366 and 0.709 mg RP036221/L (geometric mean measured); due to the poor solubility of the test item in water, test item concentrations were obtained using Water Accommodated Fractions (WAFs); the WAF of a loading of 50 mg RP036221/L was used as the highest test item concentration (stock solution); the lower concentrations were prepared by sequential dilution with the test medium in order to prepare a series of five test item concentrations with a spacing factor of 2.0 (*i.e.* 16-fold, 8-fold, 4-fold and 2-fold dilutions of the WAF of the highest test concentration were prepared).

Test conditions: 250 mL glass flasks, test volume: 100 mL; AAP nutrient solution; pH 7.14 - 7.30 at test initiation and pH 7.30 - 7.97 at test termination; temperature: 21.9 °C - 22.8 °C; initial cell densities: 1 x 10^4 cells/mL; continuous light at 6016 lux - 6172 lux, continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using an LC method with DAD detection.

Statistics: Descriptive statistics, probit analysis for determination of EC_x values for growth rate and yield, Welch-t test with Bonferroni-Holm Adjustment for determination of the NOEC 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. At test initiation, the following initial test item concentrations were measured: 0.042, 0.083, 0.177, 0.375 and 0.747 mg RP036221/L. Measured values at test termination ranged from 83.33% to 95.47% of initial concentrations. Therefore, the concentrations of the test item were stable under test conditions. Because of the poor solubility of the test item, endpoint values are based on geometric mean measured test item concentrations.

Biological results: No morphological effects on algae were observed in the control and at any of the test item concentrations tested. Statistically significant effects on algal growth rate and yield compared to the control were observed in all test item treatments after 72 hours of exposure (Welch-t test with Bonferroni-Holm Adjustment, $\alpha = 0.05$). The results are summarized in Table 8.2.6.2-4.

Table 8.2.6.2-4: Effect of RP 36221 (metabolite of iprodione) on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration (using WAFs)	**16-fold diluted**	**8-fold diluted**	**4-fold diluted**	**2-fold diluted**	**stock solution (WAF of a loading of 50 mg/L)**
Concentration [mg/L] (geometric mean measured)	**0.038**	**0.077**	**0.166**	**0.366**	**0.709**
Inhibition in 72 h (growth rate) [%]	3.5 *	5.2 *	14.6 *	39.3 *	55.7 *
Inhibition in 72 h (yield) [%]	15.3 *	21.5 *	49.9 *	84.8 *	93.4 *
Endpoints [mg RP036221/L] (geometric mean measured)					
E_rC_{50} (72 h)	0.567 (95% confidence limits: 0.531 - 0.608)				
E_rC_{10} (72 h)	0.111 (95% confidence limits: 0.096 - 0.126)				
E_yC_{50} (72 h)	0.154 (95% confidence limits: 0.137 - 0.173)				
E_yC_{10} (72 h)	0.043 (95% confidence limits: 0.032 - 0.053)				
NOEC (72 h)	< 0.038				

* Statistically significantly different compared to the control (Welch-t test with Bonferroni-Holm Adjustment, $\alpha = 0.05$).

III. CONCLUSION

In a 72-hour algae test with *Pseudokirchneriella subcapitata* the E_rC_{50} of RP 36221 (metabolite of iprodione) was determined to be 0.567 mg/L and the E_yC_{50} was 0.154 mg/L based on geometric mean measured concentrations.

The following alga toxicity study on *Pseudokirchneriella subcapitata* performed with the metabolite RP 25040 is provided in support of the aquatic risk assessment and has not been evaluated previously. The study is required, due to new data requirements.

Report:	CA 8.2.6.2/5 Swierkot A., 2013b Reg.No. 207099 (metabolite of BAS 610 F, Iprodione, RP025040) - Pseudokirchneriella subcapitata SAG 61.81 - Growth inhibition test 2013/1000222
Guidelines:	OECD 201 (2006)
GLP:	yes (certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Executive Summary

In a 72-hour static toxicity laboratory study, the effect of RP 25040 (metabolite of iprodione) on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 0 (control), 0.05, 0.14, 0.41, 1.23, 3.70, 11.11, 33.33 and 100 mg RP 25040/L. Assessment of growth was conducted 24 h, 48 h and 72 h after test initiation.

The biological results are based on nominal concentrations. No morphological effects on algae were observed in the control and at concentrations up to and including 33.33 mg RP 25040/L. At the highest tested concentration of 100 mg/L, 44.44% of the algal cells were rod-shaped. Statistically significant effects on algal growth rate and yield compared to the control were observed at the three highest test item concentrations after 72 hours of exposure.

In a 72-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} of RP 25040 (metabolite of iprodione) was determined to be 97.64 mg/L and the E_yC_{50} was 18.41 mg/L based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: RP 25040 (Reg. No. 207 099, metabolite of iprodione), batch no. TV2840/F, purity: 99.6%.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz); specification: SAG 61.81; stock obtained from "Sammlung von Algenkulturen", Göttingen, Germany.

Test design: Static system (72 hours); 8 test concentrations with 3 replicates for each plus a control with 6 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours, NOEC.

Test concentrations: Control, 0.05, 0.14, 0.41, 1.23, 3.70, 11.11, 33.33 and 100 mg RP 25040/L (nominal).

Test conditions: 250 mL glass flasks, test volume: 100 mL; AAP nutrient solution; pH 7.16 - 7.32 at test initiation and pH 7.29 - 8.14 at test termination; temperature: 21.9 °C - 22.8 °C; initial cell densities: 1 x 10^4 cells/mL; continuous light at 6868 lux - 6966 lux, continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a liquid chromatography method with DAD detection.

Statistics: Descriptive statistics, probit analysis for determination of EC_x values for growth rate and yield, Welch-t test with Bonferroni-Holm Adjustment for determination of the NOEC 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. At test initiation, concentrations in the samples of up to and including 0.14 mg RP 25040/L could not be determined, as test item concentrations were below the limit of detection. The concentration in the 0.41 mg/L treatment was below the limit of quantification. Mean measured values for RP 25040 in the samples of the test item concentrations of 1.23 mg RP 25040/L and higher ranged from 94.86% to 98.26% of nominal. At test termination, concentrations in the samples of up to and including 0.41 mg RP 25040/L were below the limit of detection and the concentration in the 1.23 mg/L treatment was below the limit of quantification. Mean measured values for RP 25040 in the samples of the test item concentrations of 3.70 mg RP 25040/L and higher were between 29.43% and 86.61% of nominal. As initially measured concentrations in the treatments at NOEC level and above (*i.e.* 1.23 mg RP 25040/L and higher concentration) confirmed correct application of the test substance, the following biological results are based on nominal concentrations.

Biological results: No morphological effects on algae were observed in the control and at concentrations up to and including 33.33 mg RP 25040/L. At the highest tested concentration of 100 mg/L, 44.44% of the algal cells were rod-shaped. Statistically significant effects on algal growth rate and yield compared to the control were observed at the three highest test item concentrations after 72 hours of exposure (Welch-t test with Bonferroni-Holm Adjustment, α = 0.05). The results are summarized in Table 8.2.6.2-5.

Table 8.2.6.2-5: Effect of RP 25040 (metabolite of iprodione) on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	**0.05**	**0.14**	**0.41**	**1.23**	**3.70**	**11.11**	**33.33**	**100**
Inhibition in 72 h (growth rate) [%] [#]	-0.1	0.0	0.5	3.0	3.5	8.9 *	17.4 *	52.0 *
Inhibition in 72 h (yield) [%] [#]	-0.4	0.2	2.2	13.7	16.1	36.1 *	58.2 *	93.3 *
Endpoints [mg RP 25040/L] (nominal)								
E_rC_{50} (72 h)	97.64 (95% confidence limits: 88.10 - 110.21)							
E_rC_{10} (72 h)	17.98 (95% confidence limits: 14.23 - 21.53)							
E_yC_{50} (72 h)	18.41 (95% confidence limits: 14.76 - 23.10)							
E_yC_{10} (72 h)	2.18 (95% confidence limits: 1.23 - 3.24)							
NOE_rC / NOE_yC (72 h)	3.70							

[#] Negative values indicate stimulated growth compared to the control.
* Statistically significantly different compared to the control (Welch-t test with Bonferroni-Holm Adjustment, α = 0.05).

III. CONCLUSION

In a 72-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} of RP 25040 (metabolite of iprodione) was determined to be 97.64 mg/L and the E_yC_{50} was 18.41 mg/L based on nominal concentrations.

The following alga toxicity study on *Pseudokirchneriella subcapitata* performed with the metabolite RP 032596 is provided in support of the aquatic risk assessment and has not been evaluated previously. The study is required, due to new data requirements.

Report:	CA 8.2.6.2/6 Rzodeczko H., 2013a Reg.No. 85831 (metabolite of BAS 610 F, Iprodione, RP032596) - Pseudokirchneriella subcapitata SAG 61.81 - Growth inhibition test 2013/1000224
Guidelines:	OECD 201 (2006)
GLP:	yes (certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Executive Summary

In a 72-hour static toxicity laboratory study, the effect of RP 032596 (metabolite of iprodione) on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 0 (control), 0.30, 0.95, 3.05, 9.77, 31.25 and 100 mg RP 032596/L. Assessment of growth was conducted 24 h, 48 h and 72 h after test initiation.

The biological results are based on nominal concentrations. No morphological effects on algae were observed in the control group and at any of the test item concentrations tested. Statistically significant effects on algal growth rate and yield compared to the control were observed at all test item concentrations except for the lowest concentration after 72 hours of exposure.

In a 72-hour algae test with *Pseudokirchneriella subcapitata*, the E_rC_{50} of RP 032596 (metabolite of iprodione) was determined to be 9.25 mg/L and the E_yC_{50} was 2.40 mg/L based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: RP 032596 (Reg. No. 85 831, metabolite of iprodione), batch no. L33-79, purity: 99.8%.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz); specification: SAG 61.81; stock obtained from "Sammlung von Algenkulturen", Göttingen, Germany.

Test design: Static system (72 hours); 6 test concentrations with 3 replicates for each plus a control with 6 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours, NOEC.

Test concentrations: Control, 0.30, 0.95, 3.05, 9.77, 31.25 and 100 mg RP 032596/L (nominal).

Test conditions: 250 mL glass flasks; test volume: 100 mL; AAP nutrient solution; pH 7.34 - 7.48 at test initiation and pH 7.10 - 8.46 at test termination; temperature: 22.3 °C - 23.3 °C; initial cell densities: 1 x 10^4 cells/mL; continuous light at 7050 lux - 7490 lux, continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a liquid chromatography method with DAD detection.

Statistics: Descriptive statistics, probit analysis for determination of EC_x values for growth rate and yield, Williams Multiple Sequential t-test Procedure and Dunnett's Multiple t-test for determination of the NOEC 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 values for RP 032596 ranged from 91.9% to 102.0% of nominal at test initiation and from 76.0% to 85.0% of nominal at test termination. As initially measured concentrations confirmed correct application of the test substance, the following biological results are based on nominal concentrations.

Biological results: No morphological effects on algae were observed in the control group and at any of the test item concentrations tested. Statistically significant effects on algal growth rate and yield compared to the control were observed at all test item concentrations except for the lowest concentration after 72 hours of exposure (Williams Multiple Sequential t-test for growth rate data and Dunnett's Multiple t-test Procedure for yield data; both with $\alpha = 0.05$). The results are summarized in Table 8.2.6.2-6.

Table 8.2.6.2**-6: Effect of RP 032596 (metabolite of iprodione) on the growth of the green alga *Pseudokirchneriella subcapitata***

Concentration [mg/L] (nominal)	**Control**	**0.30**	**0.95**	**3.05**	**9.77**	**31.25**	**100**
Inhibition in 72 h (growth rate) [%]	--	0.3	4.9 *	18.0 *	45.6 *	101.3 *, #	119.1 *, #
Inhibition in 72 h (yield) [%]	--	1.6	21.2 +	58.1 +	89.5 +	100.0 +	100.5 +, #
	Endpoints [mg RP 032596/L] (nominal)						
E_rC_{50} (72 h)	9.25 (95% confidence limits: 8.05 - 10.63)						
E_rC_{10} (72 h)	2.77 (95% confidence limits: 1.95 - 3.54)						
E_yC_{50} (72 h)	2.40 (95% confidence limits: 2.31 - 2.50)						
E_yC_{10} (72 h)	0.57 (95% confidence limits: 0.53 - 0.62)						
NOEC (72 h)	0.30						

* Statistically significantly different compared to the control (Williams Multiple Sequential t-test Procedure, $\alpha = 0.05$).
\+ Statistically significantly different compared to the control (Dunnett's Multiple t-test Procedure, $\alpha = 0.05$).
\# The values of inhibition were calculated using the original raw data on algae cell densities The values higher than 100% were not aligned to a specific value (only the original raw data were used) but can be recognized as 100% of inhibition.

III. CONCLUSION

In a 72-hour algae test with *Pseudokirchneriella subcapitata*, the E_rC_{50} of RP 032596 (metabolite of iprodione) was determined to be 9.25 mg/L and the E_yC_{50} was 2.40 mg/L based on nominal concentrations.

CA 8.2.7 Effects on aquatic macrophytes

The following toxicity study performed with iprodione is provided for the sake of completeness and has not been evaluated previously.

Report:	CA 8.2.7/1 Sowig P., 2002a Duckweed (Lemna gibba G3) growth inhibition test - Iprodione; substance, technical C022577
Guidelines:	EPA 123-2, ASTM E 1415-91, OECD
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Landwirtschaft und Forsten, Wiesbaden)

Executive Summary

In a 7-day static toxicity laboratory study, the effect of iprodione on the growth of the duckweed *Lemna gibba* was investigated. The following nominal concentrations were applied: 1.0, 1.8, 3.2, 5.6, 10.0 and 18.0 mg iprodione/L (corresponding to time-weighted average concentrations of 0.65, 1.24, 2.25, 3.98, 6.97 and 12.64 mg/L). Additionally, a solvent (acetone) control and a dilution water control were set up. Assessment of plant growth and other effects was conducted 3, 5 and 7 days after test initiation. Percent growth inhibition relative to the control was calculated for each test concentration based upon growth rates for the parameter frond number and based upon biomass for the parameter plant dry weight.

The biological results are based on time weighted average concentrations. The duckweed population in the control vessels showed sufficient growth. No morphological effects were observed at the two lowest test item concentrations of 0.65 and 1.24 mg iprodione/L. At the test item concentration of 2.25 mg a.s./L, yellow fronds were observed in two of the three replicates at day 7. Since these symptoms were not found at any higher test item concentrations, they are not considered to be dose-related. No statistically significant effects on plant growth rate (based on frond no.) compared to the solvent control were observed at any test item concentration tested. Statistically significant differences (increase) in development of biomass (based on plant dry weight) were detected at the test item concentrations of 3.98 and 6.97 mg a.s./L. However, this enhanced growth in comparison to the solvent control in terms of biomass increase is not regarded as an adverse effect.

In a 7-day aquatic-plant test with *Lemna gibba,* the E_rC_{50} of iprodione based on frond numbers and the E_bC_{50} based on dry weight were both > 12.64 mg/L (time weighted average).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Iprodione (BAS 610 F; AE F062470; Reg. no.: 101 169); purity: 97.4%.

B. STUDY DESIGN

Test species: Duckweed (*Lemna gibba* G3); cultures maintained in-house; stock obtained from "Plant Hormone Laboratory, United States Department of Agriculture", Beltsville, MD, USA.

Test design: Semi-static system; test duration 7 days; water renewal on day 3 and 5; 6 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 3, 5 and 7.

Endpoints: EC_{50} and NOEC with respect to growth rate and biomass after exposure over 7 days.

Test concentrations: Control, solvent control (0.5 mL acetone/L), 1.0, 1.8, 3.2, 5.6, 10.0 and 18.0 mg iprodione/L (nominal); corresponding to time-weighted average concentrations of 0.65, 1.24, 2.25, 3.98, 6.97 and 12.64 mg/L.

Test conditions: 300 mL glass beakers, test volume: 150 mL, 20x-AAP nutrient medium, pH 7.6 - 7.7 at test initiation and pH 7.2 - 8.9 at test termination; oxygen content: 1.5 mg/L - 9.4 mg/L, conductivity: 973 µS/cm - 1657 µS/cm, water temperature: 23.0 °C - 23.5 °C, continuous light, light intensity: about 107 - 115 $\mu E*m^{-2}*s^{-1}$.

Analytics: Analytical verification of the test item was conducted using an HPLC-method.

Statistics: Descriptive statistics, Duncan’s Multiple Range test for determination of the NOEC value.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in freshly prepared and aged test solutions for each test concentration. Measured values for iprodione ranged from 86.9% to 99.5% of nominal in fresh solutions. Analyses of aged solution showed test substance concentrations between 46.5% and 51.0% of nominal. Time-weighted average (TWA) concentrations in all treatments were between 65.1% and 71.1% of nominal. Since the TWA concentrations were below 80% of nominal at the lowest and highest treatment level the biological results were based on time-weighted average concentrations.

Biological results: The duckweed population in the control vessels showed sufficient growth, increasing from 12 fronds per vessel to an average of 174 fronds (control) and 205 (solvent control) fronds per vessel after 7 days, corresponding to a 14.5x and 17.1x multiplication, respectively. The dry weight increased from 1.8 mg to an average of 25.5 mg per vessel in the control and 40.4 mg per vessel in the solvent control, respectively, at test termination. No morphological effects were observed at the two lowest test item concentrations of 0.65 and 1.24 mg iprodione/L. At the test item concentration of 2.25 mg a.s./L, yellow fronds were observed in two of the three replicates at day 7. Since these symptoms were not found at any higher test item concentrations, they are not considered to be dose-related. Since there was a statistically significant difference between the untreated control and the solvent control (Duncan's Multiple Range test, $\alpha = 0.05$), the data from solvent groups were used for statistical evaluation of treatment related effects. No statistically significant effects on plant growth rate (based on frond no.) compared to the solvent control were observed at any test item concentration tested (Duncan's Multiple Range test, $\alpha = 0.05$). Statistically significant differences (increase) in development of biomass (based on plant dry weight) were detected at the test item concentrations of 3.98 and 6.97 mg a.s./L (Duncan's Multiple Range test, $\alpha = 0.05$). However, this enhanced growth in comparison to the solvent control in terms of biomass increase is not regarded as an adverse effect.

Effects on growth rate and development of biomass are summarized in Table 8.2.7-1.

Table 8.2.7-1: Effect of iprodione on the growth of duckweed *Lemna gibba*

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**1.0**	**1.8**	**3.2**	**5.6**	**10.0**	**18.0**
Concentration [mg a.s./L] (time-weighted average)	**--**	**--**	**0.65**	**1.24**	**2.25**	**3.98**	**6.97**	**12.64**
Inhibition after 7 d [%] # (growth rate based on frond no.)	--	--	-0.31	-1.53	2.24	-3.56	-2.38	0.10
Inhibition after 7 d [%] # (biomass based on dry weight)	--	--	-2.76	5.95	3.88	-22.78 *	-30.89 *	-20.88
Endpoints [mg iprodione/L] (time weighted average)								
E_rC_{50} (7 d) based on frond no.	> 12.64							
E_bC_{50} (7 d) based on dry weight	> 12.64							
NOEC (7 d)	≥ 12.64							

Negative values indicate stimulated growth compared to the pooled control.

* Statistically significant differences compared to the solvent control (Duncan's Multiple Range test, $\alpha = 0.05$).

III. CONCLUSION

In a 7-day aquatic-plant test with *Lemna gibba,* the E_rC_{50} of iprodione based on frond numbers and the E_bC_{50} based on dry weight were both > 12.64 mg/L (time weighted average).

CA 8.2.8 Further testing on aquatic organisms

No further studies required; thus, this point is not addressed *via* new toxicity studies.

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CA 8.3 Effects on arthropods

CA 8.3.1 Effects on bees

Table 8.3-1 Toxicity of iprodione to arthropods

Substance	Test species	Endpoint	Value	Reference	Study EU agreed?
iprodione	honeybee	24 h acute oral LD_{50}	> 25 µg a.s./bee	Mead-Briggs, R014674	Yes [1)]
		24 h acute contact LD_{50}	> 200 µg a.s./bee		
	honeybee	48 h acute oral LD_{50}	> 100 µg a.s./bee	Schmitzer 2004/1020895	No, new study
		48 h acute contact LD_{50}	> 100 µg a.s./bee		
	honeybee	10 d chronic	**ongoing**	2014/1000622	No, new study
	honeybee	7 d larvae	**ongoing**	2014/1000621	No, new study

1) In the risk assessment, endpoints obtained in the 48 h acute contact and oral study replace the endpoints from the 24 h study.

CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

Report: CA 8.3.1.1.1/1
Schmitzer S., 2004a
Effects of BAS 610 F (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
2004/1020895

Guidelines: OECD 213, OECD 214, Recent recommendations of the ICPBR group (Avignon France 1999)

GLP: Yes
(certified by Hessisches Ministerium fuer Umwelt, Landwirtschaft und Forsten, Wiesbaden)

Executive Summary

In a limit test, young adult worker bees (Apis mellifera L.) were exposed to BAS°610°F. The toxicity of the test product was determined at a concentration of 100.0 µg BAS°610°F/bee, which resulted in the same uptake. Additionally, honeybees were treated with dimethoate as reference item at concentrations ranging from 0.04 to 0.30 µg dimethoate/bee and with water and solvent controls. Mortality and behavioral effects were assessed after 4, 24 and 48 hours.

After 48 hours of oral exposure, no mortality was observed in the solvent control (acetone/sugar) whereas 2.0 % mortality occurred in the water/sugar control and 6.0 % in the test item concentration. One of the 50 bees showed behavioral impairments and was found dead after 24 hours. No more behavioral abnormalities occurred afterwards until test end (48 hours).

In an acute oral toxicity study with BAS°610 F on honeybees, the LD_{50} value (48 h) was determined to be > 100.0 µg a.s./bee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS°610°F, batch no. FF 16009, content: iprodione (BAS°610°F, Reg. No. 101°169): 97.8% (analyzed).

B. STUDY DESIGN

Test species: Honeybee (Apis mellifera L.), young adult worker bees, age: 4-6 weeks, derived from a healthy and queen-right colony, source: bred by IBACON; collected in the morning of use and kept under test conditions.

Test design: In a 48 hour oral limit test, adult worker bees of Apis mellifera were exposed orally to BAS°610°F via food (50% aqueous sugar/acetone solution). In total, 7 treatment groups were set up (1 concentration of the test item, water/sugar control, acetone/sugar control and 4 concentrations of the reference item) with 5 replicates for the control, the test item treatment and the reference item, each containing 10 bees per replicate. Assessment of bee mortality and behavioral effects was done after 4, 24 and 48 hours.

Endpoints: Mortality, behavioral impairments, determination of LD50.

Reference item: BAS 152 11 I (dimethoate, 400 g/L nominal).

Test concentrations: Control: water control (water/sugar solution), solvent control (acetone/sugar solution); BAS°610°F: 100 µg a.s./bee (nominal) resulting in an actual uptake of 100.0 µg a.s./bee in 50% aqueous sugar/acetone solution; reference item: 0.04, 0.08, 0.15 and 0.30 µg dimethoate/bee.

Test conditions: Temperature: 25°C; relative humidity: 58°% - 72 %; photoperiod: 24 h darkness; food: Apiinvert (30°% saccharose, 31°% glucose, 39°% fructose), ad libitum.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

After 48 hours of oral exposure, no mortality was observed in the solvent control (acetone/sugar) whereas 2.0 % mortality occurred in the water/sugar control and 6.0 % in the test item concentration. One of the 50 bees showed behavioral impairments and was found dead after 24 hours. No more behavioral abnormalities occurred afterwards until test end (48 hours). The results are summarized in Table 8.3.1.1.1-1.

Table 8.3.1.1.1-1: Toxicity of BAS°610°F to honeybees (*Apis mellifera*) in an oral toxicity test

Uptake of test item	**Mean mortality [%]**	
[µg BAS°610°F/bee]	**24 h**	**48 h**
water control	0.0	2.0
solvent control	0.0	0.0
100.0	4.0	6.0
	Endpoint (nominal)	
LD_{50} (48 h)	> 100.0 µg a.s./bee	

The LD50 value (24 h) for the reference item was 0.15 µg dimethoate/bee (95% confidence limits: 0.13 – 0.16 µg dimethoate/bee) in the oral toxicity test.

III. CONCLUSION

In an acute oral toxicity study with BAS°610 F on honeybees, the LD_{50} value (48 h) was determined to be > 100.0 µg a.s./bee.

CA 8.3.1.1.2 Acute contact toxicity

Report:	CA 8.3.1.1.2/1 Schmitzer S., 2004a Effects of BAS 610 F (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory 2004/1020895
Guidelines:	OECD 213, OECD 214, Recent recommendations of the ICPBR group (Avignon France 1999)
GLP:	Yes (certified by Hessisches Ministerium fuer Umwelt, Landwirtschaft und Forsten, Wiesbaden)

Executive Summary

In a limit test, young adult worker bees (*Apis mellifera* L.) were exposed to BAS°610°F. The toxicity of the test product was determined at a concentration of 100.0 µg BAS°610°F/bee. Additionally, honeybees were treated with dimethoate as reference item at concentrations ranging from 0.10 to 0.30 µg dimethoate/bee and with water and solvent controls. Mortality and behavioral effects were assessed after 4, 24 and 48 hours.

After 48 hours of contact exposure, 2.0 % mortality occurred in both control groups compared to 8.0 % in the test item concentration. No test item induced behavioral effects were observed at any time in the contact test.

In an acute contact toxicity study with BAS°610°F on honeybees, the LD_{50} value (48 h) was determined to be > 100.0 µg a.s./bee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS°610°F, batch no. FF 16009, content: iprodione (BAS°610°F, Reg. No. 101°169): 97.8% (analyzed).

B. STUDY DESIGN

Test species: Honeybee (Apis mellifera L.), young adult worker bees, age: 4-6 weeks, derived from a healthy and queen-right colony, source: bred by IBACON; collected in the morning of use and kept under test conditions.

Test design: In a 48 hour contact limit test, adult worker bees of Apis mellifera were exposed to one concentration of BAS°610°F in solvent (solvent = acetone) placed on the bee thorax using a Burkard-Applicator. In total, 7 treatment groups were set up (1 concentration of the test item, water with 1% Adhäsit with anesthetization as control, acetone with anesthetization as control and 4 concentrations of the reference item) with 5 replicates for the control, the test item treatment and the reference item, each containing 10 bees per replicate. Assessment of bee mortality and behavioral effects was done after 4, 24 and 48 hours.

Endpoints: Mortality, behavioral impairments, determination of LD50.

Reference item: BAS 152 11 I (dimethoate, 400 g/L nominal).

Test concentrations: Control: water control (water with 1% Adhäsit with anesthetization), solvent control (acetone with anesthetization); BAS°610°F: 100 µg a.s./bee (nominal); reference item: 0.10, 0.15, 0.20 and 0.30 µg dimethoate/bee (nominal).

Test conditions: Temperature: 25°C; relative humidity: 58°% - 72 %; photoperiod: 24 h darkness; food: Apiinvert (30°% saccharose, 31°% glucose, 39°% fructose), ad libitum.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

After 48 hours of contact exposure, 2.0 % mortality occurred in both control groups compared to 8.0 % in the test item concentration. No test item induced behavioral effects were observed at any time in the contact test. The results are summarized in Table 8.3.1.1.2-1.

Table 8.3.1.1.2-1: Toxicity of BAS°610°F to honeybees (*Apis mellifera*) in a contact toxicity test

Treatment [µg BAS°610°F/bee]	Mean mortality [%]	
	24 h	48 h
water control	0.0	2.0
solvent control	0.0	2.0
100.0	4.0	8.0
	Endpoint (nominal)	
LD_{50} (48 h)	> 100.0 µg a.s./bee	

The LD50 value (24 h) for the reference item was 0.27 µg dimethoate/bee (95% confidence limits: 0.21 – 0.34 µg dimethoate/bee) for O. lignaria and 0.15 µg dimethoate/bee (95% confidence limits: 0.13 – 0.17 µg dimethoate/bee) for A. mellifera in the oral toxicity test.

III. CONCLUSION

In an acute contact toxicity study with BAS°610°F on honeybees, the LD_{50} value (48 h) was determined to be > 100.0 µg a.s./bee.

CA 8.3.1.2 Chronic toxicity to bees

Report: CA 8.3.1.2/1
Effect of BAS 610 F on larval development of honey bees
2014/1000622
Guidelines:
GLP: no

New study requirement, study will be conducted in 2014 and submitted when finalized (expected submission Q4 2014, DocID 2014/1000622).

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Report: CA 8.3.1.3/1
Effect of BAS 610 F on larval development of honey bees
2014/1000621
Guidelines:
GLP: no

New study requirement, study will be conducted in 2014 and submitted when finalized (expected submission Q4 2014, DocID 2014/1000621).

CA 8.3.1.4 Sub-lethal effects

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

Table 8.4-1 Toxicity to non-target soil meso- and macrofauna of iprodione and relevant metabolites

Substance	Species	Endpoint	Value [mg/kg dry soil]	Reference	Study EU agreed?
iprodione	*Eisenia fetida*	LC_{50}	> 1000	Handley and Wetton, R014575	Yes
iprodione	*Eisenia fetida*	LC_{50}	> 1000	Witte, 2010/1075819	No, new study[1)]
iprodione	*Eisenia fetida*	NOEC	> 1000	Mc Elligott, R014682	Yes
RP 30228	*Eisenia fetida*	NOEC	≥ 1000	Odin-Feurtet, R014680	Yes
RP 32596	*Eisenia fetida*	NOEC	≥ 100	Lührs, R014341	Yes
RP 36221	*Eisenia fetida*	NOEC	≥ 43.4	Schöbinger, 2012/1202314	No, new study
RP 25040	*Eisenia fetida*	NOEC	≥ 100	Friedrich 2014/1000501	No, new study

1) summary can be found in chapter 8.7

CA 8.4.1 Earthworms – sub-lethal effects

Report:	CA 8.4.1/1 Schoebinger U., 2013a Sublethal toxicity of Reg.No. 5079618 (metabolite of BAS 610 F, RP 36221) to the earthworm Eisenia fetida in artificial soil with 5% peat 2012/1202314
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 RP 36221, a metabolite of iprodione, on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in a 56-day reproduction study. Five concentrations (2.71, 5.42, 10.8, 21.7 and 43.4 mg RP 36221/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 43.4 mg RP 36221/kg dry soil. Body weight and reproduction rate of earthworms exposed to RP 36221 was not statistically significantly different compared to the control up to the highest concentration tested. Neither behavioural abnormalities nor effects on feeding activity were observed in any of the treatment groups.

In a 56-day earthworm reproduction study with RP 36221 (a metabolite of iprodione) on earthworms (*Eisenia fetida*), the NOEC for mortality, biomass, reproduction and feeding activity was determined to be ≥ 43.4 mg RP 36221/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: RP 36221 (metabolite of iprodione, Reg. No. 5 079 618) batch no. L80-74; purity: 92.2%

B. STUDY DESIGN

Test species: Earthworm (Eisenia fetida), adult worms (with clitellum); weight: 383 mg – 574 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 (5% peat only); 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: NOEC; effects on mortality, weight change, reproduction rate, feeding activity.

Reference item: Twist WP (carbendazim, 583 g/kg). The effects of the reference item were investigated in a separate study.

Test concentrations: Control, 2.5, 5.0, 10, 20 and 40 mg test item/kg dry soil (analyzed); corresponding to 2.71, 5.42, 10.8, 21.7 and 43.4 mg test item/kg dry soil (nominal).

Test conditions: Artificial soil according to OECD 222 (with reduced content of peat: 5%); pH 6.46 - 6.55 at test initiation, pH 7.21 - 7.45 at test termination; maximum water holding capacity (WHC): 56.7% - 58.6% at test initiation, 58.1% - 69.9% of WHC at test termination; temperature: 19.1 °C - 20.3 °C; photoperiod: 16 h light : 8 h dark, light intensity: 400 - 750 lux; food: horse manure.

Statistics: Descriptive statistics, Dunnett's t-test for reproduction data ($\alpha = 0.05$).

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 43.4 mg RP 36221/kg dry soil. Body weight and reproduction rate of earthworms exposed to RP 36221 was not statistically significantly different compared to the control (Dunnett's-t-test, $\alpha = 0.05$) up to the highest concentration tested. Neither behavioural abnormalities nor effects on feeding activity were observed in any of the treatment groups. The results are summarized below (see Table 8.4.1-1).

Table 8.4.1-1: Effects of RP 36221, a metabolite of iprodione, on earthworms (*Eisenia fetida)* in a 56-day reproduction study

RP 36221 [mg/kg dry soil] [1)]	**Control**	**2.71**	**5.42**	**10.8**	**21.7**	**43.4**
Mortality (day 28) [%]	0.0	0.0	0.0	0.0	0.0	0.0
Weight change (day 28) [%]	3.84	10.9	17.8	10.7	0.566	7.07
Mean no. of juveniles (day 56)	48.7 [2)]	54.0	36.8	49.5	67.5	54.5
Reproduction (day 56) [% deviation from control]	--	10.9	-24.2	1.6	38.6	11.9
Feeding activity [%]	100.0	100.0	100.0	100.0	100.0	100.0
Endpoints [mg RP 36221/kg dry soil]						
$NOEC_{mortality, weight}$ (day 28)	≥ 43.4					
$NOEC_{reproduction}$ (day 56)	≥ 43.4					

1) based on nominal contents
2) value based on 7 replicates

III. CONCLUSION

In a 56-day earthworm reproduction study with RP 36221 (a metabolite of iprodione) on earthworms (Eisenia fetida), the NOEC for mortality, biomass, reproduction and feeding activity was determined to be ≥ 43.4 mg RP 36221/kg dry soil.

Report:	CA 8.4.1/2 Friedrich S., 2014a Sublethal toxicity of Reg.No. 207099 (metabolite of BAS 610 F, Iprodione) to the earthworm Eisenia fetida in artificial soil 2014/1000501
Guidelines:	OECD 222 (2004)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of RP 25040, a metabolite of iprodione, on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in a 56-day reproduction study. Five concentrations (6.25, 12.5, 25, 50 and 100 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 the mortality was about 0% - 5.0% in the test item treatment groups and 1.3% in the control group. Mortality, body weight and reproduction rate of earthworms exposed to RP 25040 was not statistically significantly different compared to the control up to and including the highest concentration tested. Neither behavioural abnormalities nor effects on feeding activity were observed in any of the treatment groups.

In a 56-day earthworm reproduction study with RP 25040 (a metabolite of iprodione) on earthworms (*Eisenia fetida*), the NOEC for mortality, biomass, reproduction and feeding activity was determined to be ≥ 100 mg RP 25040/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: RP 25040 (metabolite of iprodione, Reg. No. 207 099) batch no. L80-192; purity: 100.0 % (tolerance ± 1.0 %)

B. STUDY DESIGN

Test species: Earthworm (*Eisenia fetida*), adult worms (with clitellum); weight: 332 mg – 528 mg), age: approximately 4 months old; source: 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 test vessels, before the earthworms were introduced on the top of the soil. Assessment of worm mortality, behavioural effects and biomass development after 28 days; assessment of reproduction rate (number of offspring) after another 28 days (56 days after application).

Endpoints: NOEC; 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.

Test concentrations: Control, 6.25, 12.5, 25, 50 and 100 mg test item/kg dry soil.

Test conditions: Artificial soil according to OECD 222 (with 10% peat); pH 6.08 - 6.15 at test initiation, pH 5.77 - 5.86 at test termination; water content: 56.4% - 56.5% of maximum water holding capacity (WHC) at test initiation, 55.9% of WHC at test termination; temperature: 18.0 °C - 21.1 °C; photoperiod: 16 h light : 8 h dark, light intensity: 570 lux; food: horse manure.

Statistics: Descriptive statistics, Fisher's Exact Binomial Test for mortality ($\alpha = 0.05$, one-sided greater), Williams-t-test for weight change and reproduction ($\alpha = 0.05$, one-sided smaller).

II. RESULTS AND DISCUSSION

After 28 days of exposure the mortality was about 0% - 5.0% in the test item treatment groups and 1.3% in the control group. Mortality, body weight and reproduction rate of earthworms exposed to RP 25040 was not statistically significantly different compared to the control (Fisher`s Exact Binominal test or Williams-t-test, $\alpha = 0.05$) up to and including the highest concentration tested. Neither behavioural abnormalities nor effects on feeding activity were observed in any of the treatment groups. The results are summarized below (see Table 8.4.1-2).

Table 8.4.1-2: Effects of RP 25040, a metabolite of iprodione, on earthworms (*Eisenia fetida)* in a 56-day reproduction study

RP 25040 [mg/kg dry soil]	**Control**	**6.25**	**12.5**	**25**	**50**	**100**
Mortality (day 28) [%]	1.3	0.0	0.0	2.5	5.0	0.0
Weight change (day 28) [%]	31.9	35.4	29.3	32.4	31.1	28.5
Mean no. of juveniles (day 56)	130.5	124.8	140.8	124.3	129.8	113.5
Reproduction in [%] of control (day 56)	100	95.6	107.9	95.2	99.4	87.0
Feeding activity [%]	100.0	100.0	100.0	100.0	100.0	100.0
Endpoints [mg RP 25040/kg dry soil]						
$NOEC_{mortality, weight}$ (day 28)	≥ 100					
$NOEC_{reproduction}$ (day 56)	≥ 100					

III. CONCLUSION

In a 56-day earthworm reproduction study with RP 25040 (a metabolite of iprodione) on earthworms (*Eisenia fetida*), the NOEC for mortality, biomass, reproduction and feeding activity was determined to be ≥ 100 mg RP 25040/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

Not triggered; no new studies are available.

CA 8.5 Effects on nitrogen transformation

Table 8.5-1: Toxicity to nitrogen transformation of iprodione and relevant metabolites

Substance	Endpoint	NOEC [mg/kg dry soil]	Reference	Study EU agreed?
iprodione	Effects on nitrogen transformation	8.0	Mallett et al., R014538	No, new study
RP 30228 (metabolite of iprodione)	Effects on nitrogen transformation	8.0	McMurray C022588	No, new study
RP 32596 (metabolite of iprodione)	Effects on nitrogen transformation	8.0	McMurray, C014354	No, new study
RP 36221 (metabolite of iprodione)	Effects on nitrogen transformation	10.0	Schulz, 2012/1202312	No, new study
RP 25040 (metabolite of iprodione)	Effects on nitrogen transformation	10.0	Schulz, 2012/1202313	No, new study

Studies are required, as they address soil metabolites.

Report:	CA 8.5/1 Mallett M.J.,Hayward J.C., 1999a A laboratory assessment of the effects of Iprodione on soil microflora respiration and nitrogen transformations according to current EU guidelines R014538
Guidelines:	EPPO Bulletin 24 (1994)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

The effect of BAS°610°F on nitrogen transformation was tested in a lucerne-enriched sandy loam / loamy sand soil. BAS°610°F was applied to samples of the soil, in a laboratory, at a test concentration of 8.0 mg/kg dry soil, equivalent to 6 kg/ha. The treated soils and untreated controls were incubated at 20 ± 2 °C in the dark for 28 days. Triplicate samples of each treatment were removed for analysis of NH_4-nitrogen, NO_2-nitrogen and NO_3-nitrogen 0, 14 and 28 days after application.

No adverse effects of BAS°610°F on nitrogen transformation could be observed at the test concentration of 8.0 mg BAS°610°F/kg dry soil after 28 days (sandy loam / loamy sand).

Based on the results of this study, BAS°610°F caused no adverse effects (< 25% deviation from control according to OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) in sandy loam / loamy sand tested at a concentration of 8.0 mg/kg dry soil after 28 days of incubation.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS°610°F, batch no. TV3015C, content: iprodione (BAS°610°F, Reg. No. 101°169): 99.7% (w/w).

B. STUDY DESIGN

Test soil: Biologically active agricultural soil: sandy loam (UK classification) / loamy sand (BBA classification), soil pH 6.5 (water), 6.1 (M KCl), Corg 1.1%, WHC: 59.5 g/100 g dry soil.

Test design: Determination of the N-transformation (NO3-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5%). Comparison of test item treated soil with a non-treated soil. NH4-nitrogen formed from organically bound nitrogen and NO3-nitrogen formed from the nitrification process was determined. Sampling scheme: 0, 14 and 28 days after treatment. Sub-samples were withdrawn from the bulk batches and subjected to the measurement.

Endpoints: Effects on the NO3-nitrogen production 0, 14 and 28 days after exposure.

Test concentrations: Control (with and without Lucerne), 8.0 mg BAS°610°F/kg dry soil (equivalent to 6 kg BAS°610°F/ha); test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm3.

Reference item: Dinoseb (purity: 99.9%). The reference item was tested at concentrations of 1 and 10 mg/kg dry soil.

Test conditions: Water content: approx. 45% of maximum water holding capacity. Soil samples were incubated at 20 ± 2 °C while stored in plastic vessels in the dark.

Statistics: Descriptive statistics. Students two sample t-test ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

No adverse effects of BAS°610°F on nitrogen transformation could be observed at the test concentration of 8.0 mg BAS°610°F/kg dry soil after 28 days (sandy loam / loamy sand). Only negligible deviations from the control of +8.3% were measured after 28 days. The results are summarized below in Table 8.5-2.

Table 8.5-2: Effects of BAS°610°F on soil micro-organisms (nitrogen transformation) on days 0, 14 and 28 of incubation

Soil (days)	Control with lucerne	Control without lucerne	8.0 mg BAS°610°F/kg dry soil	
	NO_3-N [mg/kg dry soil]		NO_3-N [mg/kg dry soil]	% Deviation from control [1)]
sandy loam / loamy sand (0 d)	17.31	15.36	17.35	+0.2
sandy loam / loamy sand (14 d)	31.95	--	34.85	< 0.26
sandy loam / loamy sand (28 d)	38.33	--	41.53	+8.3

1) Based on NO_3-nitrogen production; - = inhibition, + = stimulation.

The reference item dinoseb produced a stimulation of nitrogen transformation of +95.6% at 10 mg/kg dry soil after 28 days incubation.

III. CONCLUSION

Based on the results of this study, BAS°610°F caused no adverse effects (< 25% deviation from control according to OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) in sandy loam / loamy sand tested at a concentration of 8.0 mg/kg dry soil after 28 days of incubation.

Report:	CA 8.5/2 McMurray A., 1999a A laboratory assessment of the effects of RP 30228 on soil microflora respiration and nitrogen transformations according to current EU guidelines C022588
Guidelines:	EPPO Bulletin 24 (1994)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

The effect of RP 30228 on nitrogen transformation was tested in a lucerne-enriched loamy sand soil and sandy loam soil. RP 30228 was applied to samples of the soil at a test concentration of 6 kg/ha. Treated and untreated soils were incubated at approximately 20 ± 2 °C in the dark.

No adverse effects of RP 30228 on nitrogen transformation in soil could be observed in the test item concentration (6 kg RP 30228/ha) after 28 days in the sandy loam soil and after 42 days in the loamy sand soil. Only small deviations from the control of -11.50% were measured in the sandy loam soil after 28 days as well as of +4.13% and +0.41% in the loamy sand soil after 28 and 42 days, respectively.

Based on the results of this study, RP°30228 caused no adverse effects (< 25% deviation from control according to OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) in sandy loam / loamy sand tested at a concentration of 6 kg/ha after 28 days of incubation.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: RP 30228 (metabolite of iprodione, BAS 610 F), batch no. DP653, purity: 99.4% (w/w).

B. STUDY DESIGN

Test soil: Biologically active agricultural soils: a sandy loam (BBA classification): soil pH 7.3 (in water), Corg 1.5%, WHC 57.7 g/100 g dry soil;
a loamy sand (BBA classification): soil pH 5.9 (in water), Corg 2.7%, WHC 59.3 g/100 g dry soil

Test design: Determination of the N-transformation (NO3-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5%). Comparison of test item treated soil with a non-treated soil. NH4-nitrogen formed from organically bound nitrogen and NO3-nitrogen formed from the nitrification process was determined. Sampling scheme: 0, 14 and 28 days after treatment for the sandy loam and 0, 14, 28 and 42 days after treatment for the loamy sand. Sub-samples were withdrawn from the bulk batches and subjected to the measurement.

Endpoints: Effects on the NO3-nitrogen production 0, 14 and 28 days after exposure.

Test concentrations: Control and 6 kg/ha (applied in quartz sand that was even distributed in the test soil).

Reference item: Dinoseb. The reference item was tested at a rate of 100 L/ha in a separate study.

Test conditions: Water content: approx. 45% of maximum water holding capacity. Soil samples were incubated at 20 ± 2 °C while stored in plastic vessels in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of RP 30228 on nitrogen transformation in soil could be observed in the test item concentration (6 kg RP 30228/ha) after 28 days in the sandy loam soil and after 42 days in the loamy sand soil. Only small deviations from the control of -11.50% were measured in the sandy loam soil after 28 days as well as of +4.13% and +0.41% in the loamy sand soil after 28 and 42 days, respectively. The results are summarized below in Table 8.5-3.

Table 8.5-3: Effects of RP 30228 on soil micro-organisms (nitrogen transformation) on days 0, 14, 28 and 42 of incubation

Days	Sandy loam soil			Loamy sand soil		
	Control soil (with lucerne)	6 kg/ha		Control soil (with lucerne)	6 kg/ha	
	NO3-N [mg/kg dry soil]	NO3-N [mg/kg dry soil]	% deviation from the control [1)]	NO3-N [mg/kg dry soil]	NO3-N [mg/kg dry soil]	% deviation from the control [1)]
0	34.85	36.45	+4.59	31.17	29.83	-4.30
14	33.19	41.16	+24.01	47.10	46.71	-0.83
28	54.44	48.18	-11.50	89.57	93.27	+4.13
42	not determined			118.51	119.00	+0.41

[1)] Based on NO_3-nitrogen production; - = inhibition, + = stimulation.

In a separate study the reference item dinoseb caused inhibitions of nitrogen transformation of 70.86% after 44 days in a sandy loam soil and of 35.85% after 28 days in a loamy sand soil, both at 100 L/ha. Both values were significantly different from the controls ($\alpha = 0.05$).

III. CONCLUSION

Based on the results of this study, RP°30228 caused no adverse effects (< 25% deviation from control according to OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) in sandy loam / loamy sand tested at a concentration of 6 kg/ha after 28 days of incubation.

Report:	CA 8.5/3 McMurray A., 2001a A laboratory assessment of the effects of RP32596 (3,5-DCA) on soil microflora respiration and nitrogen transformations according to current EU-guidelines C014354
Guidelines:	EPPO Bulletin 24 (1994)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

The effect of RP 32596 on nitrogen transformation was tested in a humus sandy soil and a sandy clay soil. RP 32596 was applied to samples of the soil at a test concentration of 6 kg/ha. Treated and untreated soils were incubated at approximately 20 ± 2 °C in the dark.

No adverse effects of RP 32596 on nitrogen transformation in soil could be observed in the test item concentration (6 kg RP 32596/ha) after 28 days in the humus sandy soil and sandy clay soil. Statistically significant effects in the test item treatment compared to the control were found in the humus sandy soil after 14 days. However, after 28 days, only small deviations from the control of -7.03% were measured in the humus sandy soil and of -3.29% in the sandy clay soil.

Based on the results of this study, RP 32596 caused no adverse effects (< 25% deviation from control according to OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) in humus sandy soil / sandy clay soil tested at a concentration of 6 kg/ha after 28 days of incubation.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: RP 32596 (3,5-DCA) (metabolite of iprodione, BAS 610 F), batch no. 29027/8, purity 99.1% (w/w).

B. STUDY DESIGN

Test soil: Biologically active agricultural soils: a humus sandy soil and a sandy clay soil (BBA classification), soil characteristics:
humus sandy soil: pH 6.2 (in water), Corg 5.4%, WHC 30.17 g/100 g soil; sandy clay soil: pH 8.2 (in water), Corg 1.9%, WHC 20.50 g/100 g soil.

Test design: Determination of the N-transformation (NO3-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5%). Comparison of test item treated soil with a non-treated soil. NH4-nitrogen formed from organically bound nitrogen and NO3-nitrogen formed from the nitrification process was determined by using a three channel Flow Injection. Sampling scheme: 0, 14 and 28 days after treatment. Sub-samples were withdrawn from the bulk batches and subjected to the measurement.

Endpoints: Effects on the NO3-nitrogen production 0, 14 and 28 days after exposure.

Test concentrations: Control and 6 kg/ha (equivalent to 8.0 mg/kg dry soil, applied in quartz sand that was even distributed in the test soil).

Reference item: Dinoseb (purity: 99.9%). The reference item was tested at a rate of 56 kg/ha in a separate study.

Test conditions: Water content: approx. 55% of maximum water holding capacity. Soil samples were incubated at 20 ± 2 °C while stored in plastic vessels in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of RP 32596 on nitrogen transformation in soil could be observed in the test item concentration (6 kg RP 32596/ha) after 28 days in the humus sandy soil and sandy clay soil. Statistically significant effects in the test item treatment compared to the control were found in the humus sandy soil after 14 days ($\alpha = 0.05$). However, after 28 days, only small deviations from the control of -7.03% were measured in the humus sandy soil and of -3.29% in the sandy clay soil. The results are summarized below in Table 8.5-4.

Table 8.5-4: Effects of RP 32596 on soil micro-organisms (nitrogen transformation) on days 0, 14 and 28 of incubation

Days	Humus sandy soil			Sandy clay soil		
	Control soil (with lucerne)	6 kg/ha		Control soil (with lucerne)	6 kg/ha	
	NO3-N [mg/kg dry soil]	NO3-N [mg/kg dry soil]	% deviation from the control [1)]	NO3-N [mg/kg dry soil]	NO3-N [mg/kg dry soil]	% deviation from the control [1)]
0	13.11	12.26	-6.48	15.64	15.29	-2.24
14	32.38	28.95	-10.59 *	32.74	29.88	-8.74
28	41.85	38.91	-7.03	36.77	35.56	-3.29

1) Based on NO_3-nitrogen production; - = inhibition, + = stimulation.

* statistically significantly different from the control ($\alpha = 0.05$).

In a separate study the reference item dinoseb caused a stimulation of nitrogen transformation of 29.94% after 28 days in a silty loam soil The value was statistically significantly different from the controls ($\alpha = 0.05$).

III. CONCLUSION

Based on the results of this study, RP 32596 caused no adverse effects (< 25% deviation from control according to OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) in humus sandy soil / sandy clay soil tested at a concentration of 6 kg/ha after 28 days of incubation.

Report:	CA 8.5/4 Schulz L., 2012a Effects of Reg.No. 5079618 (metabolite of BAS 610 F, RP 36221) on the activity of soil microflora (Nitrogen transformation test) 2012/1202312
Guidelines:	OECD 216 (2000)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effect of RP 36221 on nitrogen transformation was tested in a lucerne-enriched loamy sand / sandy loam soil. RP 36221 was applied to samples of the soil at nominal test concentrations of 1.0 and 10 mg/kg dry soil. Treated and untreated soils were incubated at approximately 20 °C in the dark.

No adverse effects of RP 36221 on nitrogen transformation in soil could be observed at a test item concentration of 1.0 mg/kg dry soil 28 days after application. Only a negligible deviation from control of +10.3 % was measured at the end of the 28-day incubation period.
Due to a measured deviation of > 25% observed in the treatment group treated with 10 mg RP 36221/kg dry soil, 28 days after application, the test had to be prolonged up to day 42 after application. Only a negligible deviation from control of +23.1 % was measured at the end of the 42-day incubation period.

Based on the results of this study, RP 36221 caused no adverse effects (< 25% deviation from control according to OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) in loamy sand / sandy loam tested up to a concentration of 10 mg/kg dry soil after 42 days of incubation.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: RP 36221 (metabolite of iprodione, BAS 610 F), batch no.: L80-74, Reg. No.: 5 079 618, purity: 92.2 % (tolerance ±1.0%).

B. STUDY DESIGN

Test soil: Biologically active agricultural soil: loamy sand (DIN 4220) / sandy loam (USDA), pH 6.5, 1.45% Corg, WHC: 33.45 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. NH_4-nitrogen formed from organically bound nitrogen and NO_3-nitrogen formed from the nitrification process was determined using an Autoanalyzer (Bran and Luebbe). Sampling scheme: 0, 14, 28 and 42 days after treatment. Sub-samples were withdrawn from the bulk batches and subjected to the measurement.

Endpoints: Effects on the NO_3-nitrogen production 0, 14, 28 and 42 days after exposure.

Test concentrations: Control, treatment groups: 1.0 mg and 10 mg RP 36221/kg dry soil.

Reference item: Dinoterb (purity: 98.0% ±0.5 analyzed). The reference item was tested in a separate study at rates of 6.80, 16.00 and 27.00 mg/kg.

Test conditions: Water content: approx. 45% of maximum water holding capacity, measured water content: 14.78 - 15.48 g/100 g dry soil; pH 6.2. Soil samples were incubated at 18.9 - 21.0 °C while stored in glass flasks in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of RP 36221 on nitrogen transformation in soil could be observed at a test item concentration of 1.0 mg/kg dry soil 28 days after application. Only a negligible deviation from control of +10.3 % was measured at the end of the 28-day incubation period.
Due to a measured deviation of > 25 % observed in the treatment group treated with 10 mg RP 36221/kg dry soil, 28 days after application, the test had to be prolonged up to day 42 after application. Only a negligible deviation from control of +23.1 % was measured at the end of the 42-day incubation period. The results are summarized below in Table 8.5-5.

Table 8.5-5: Effects of RP 36221 on soil micro-organisms (nitrogen transformation) on days 0, 14, 28 and 42 of incubation

Soil (days)	Control	1.0 mg RP 36221/kg dry soil		10.0 mg RP 36221/kg dry soil	
	NO_3-N [µl/kg dry soil]	NO_3-N [µl/kg dry soil]	% Deviation from control [1)]	NO_3-N [µl/kg dry soil]	% Deviation from control [1)]
loamy sand / sandy loam (0 - 7 d)	10.03	13.40	+33.6	13.77	+37.2
loamy sand / sandy loam (0 - 14 d)	16.43	17.90	+8.9	19.80	+20.5
loamy sand / sandy loam (0 - 28 d)	23.87	26.33	+10.3	30.07	+26.0
loamy sand / sandy loam (0 - 42 d)	30.60	n.d.	--	37.67	+23.1

[1)] Based on NO_3-nitrogen production; - = inhibition, + = stimulation.
n.d.= not determined

In a separate study the reference item dinoterb caused produced a stimulation of nitrogen transformation of +40.4 %, +68.1 % and +83.5 % at 6.80 mg, 16.00 mg and 27.00 mg/kg dry soil respectively, 28 days after application.

III. CONCLUSION

Based on the results of this study, RP 36221 caused no adverse effects (< 25% deviation from control according to OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) in loamy sand / sandy loam tested up to a concentration of 10 mg/kg dry soil after 42 days of incubation.

Report:	CA 8.5/5 Schulz L., 2013a Effects of Reg.No. 207099 (metabolite of BAS 610 F, RP 25040) on the activity of soil microflora (Nitrogen transformation test) 2012/1202313
Guidelines:	OECD 216 (2000)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effect of RP 25040 on nitrogen transformation was tested in a lucerne-enriched loamy sand / sandy loam soil. RP 25040 was applied to samples of the soil at nominal test concentrations of 1.0 and 10 mg/kg dry soil. Treated and untreated soils were incubated at approximately 20 °C in the dark.

No adverse effects of RP 25040 on nitrogen transformation in soil could be observed in both test concentrations (1.0 mg/kg dry soil and 10 mg/kg dry soil) after 28 days. Only negligible deviations from the control of +10.1 % (test concentration 1.0 mg/kg dry soil) and +21.8 % (test concentration 10 mg/kg dry soil) were measured at the end of the 28-day incubation period.

Based on the results of this study, RP 25040 caused no adverse effects (< 25% deviation from control according to OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) in loamy sand / sandy loam tested up to a concentration of 10 mg/kg dry soil after 28 days of incubation.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: RP 25040 (metabolite of iprodione, BAS 610 F), batch no.: TV2840/F, Reg. No.: 207 099, purity: 99.6% (tolerance ±1.0 %)

B. STUDY DESIGN

Test soil: Biologically active agricultural soil: loamy sand (DIN 4220) / sandy loam (USDA), pH 6.5, 1.45 % Corg, WHC: 33.45 g/100 g dry soil.

Test design: Determination of the N-transformation (NO3-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5 %). Comparison of test item treated soil with a non-treated soil. NH4-nitrogen formed from organically bound nitrogen and NO3-nitrogen formed from the nitrification process was determined using an Autoanalyzer (Bran and Luebbe). Sampling scheme: 0, 14 and 28 days after treatment. ub-samples were withdrawn from the bulk batches and subjected to the measurement.

Endpoints: Effects on the NO3-nitrogen production 0, 14 and 28 days after exposure.

Test concentrations: Control, treatment groups: 1.0 mg and 10 mg RP 25040/kg dry soil.

Reference item: Dinoterb (purity: 98.0% ±0.5 analyzed). The reference item was tested in a separate study at rates of 6.80, 16.00 and 27.00 mg/kg.

Test conditions: Water content: approx. 45 % of maximum water holding capacity, measured water content: 15.23 - 16.10 g/100 g dry soil; pH 6.1 - 6.3. Soil samples were incubated at 18.9 - 21.0 °C while stored in glass flasks in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of RP 25040 on nitrogen transformation in soil could be observed in both test concentrations (1.0 mg/kg dry soil and 10 mg/kg dry soil) after 28 days. Only negligible deviations from the control of +10.1 % (test concentration 1.0 mg/kg dry soil) and +21.8 % (test concentration 10 mg/kg dry soil) were measured at the end of the 28-day incubation period. The results are summarized below in Table 8.5-6.

Table 8.5-6: Effects of RP 25040 on soil micro-organisms (nitrogen transformation) on days 0, 14 and 28 of incubation

Soil (days)	Control	1.0 mg RP 25040/kg dry soil		10.0 mg RP 25040/kg dry soil	
	NO_3-N [µl/kg dry soil]	NO_3-N [µl/kg dry soil]	% Deviation from control [1)]	NO_3-N [µl/kg dry soil]	% Deviation from control [1)]
loamy sand / sandy loam (0 - 7 d)	8.90	10.80	+21.3	12.57	+41.2
loamy sand / sandy loam (0 - 14 d)	13.63	14.03	+2.9	15.93	+16.9
loamy sand / sandy loam (0 - 28 d)	20.80	22.90	+10.1	25.33	+21.8

[1)] Based on NO_3-nitrogen production; - = inhibition, + = stimulation.

In a separate study the reference item dinoterb caused produced a stimulation of nitrogen transformation of +40.4 %, +68.1 % and +83.5 % at 6.80 mg, 16.00 mg and 27.00 mg/kg dry soil respectively, 28 days after application.

III. CONCLUSION

Based on the results of this study, RP 25040 caused no adverse effects (< 25% deviation from control according to OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) in loamy sand / sandy loam tested up to a concentration of 10 mg/kg dry soil after 28 days of incubation.

CA 8.6 Effects on terrestrial non-target higher plants

CA 8.6.1 Summary of screening data

No new studies are available.

CA 8.6.2 Testing on non-target plants

No new studies are available.

CA 8.7 Effects on other terrestrial organisms (flora and fauna)

The following studies are presented as additional information.

Report:	CA 8.7/1 Mallett M.J.,Hayward J.C., 1999a A laboratory assessment of the effects of Iprodione on soil microflora respiration and nitrogen transformations according to current EU guidelines R014538
Guidelines:	EPPO Bulletin 24 (1994)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

In a soil microbial activity study, the effects of BAS 610 F on the carbon transformation were investigated in a loamy sand / sandy loam soil. BAS 610 F was applied to samples of the soil at a test concentrations of 8.0 mg a.s./kg dry soil. Treated and untreated soils were incubated at approximately 20 ± 2 °C in the dark.

No adverse effects of BAS 610 F on carbon transformation in soil could be observed in the test item concentration (8.0 mg a.s./kg dry soil) after 28 days. Only a negligible deviation from the control of +2.6 % was measured at the end of the 28-day incubation period.

BAS 610 F (iprodione) caused no adverse effects (deviation from control < 25%, OECD 217) on the soil carbon transformation (measured as carbon dioxide production) at the end of the 28-day incubation period. The study was performed at a concentration of 8.0 mg a.s./kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS°610°F, batch no. TV3015C, content: iprodione (BAS°610°F, Reg. No. 101°169): 99.7% (w/w).

B. STUDY DESIGN

Test soil: Biologically active agricultural soil: sandy loam (UK classification) / loamy sand (BBA classification), soil pH 6.5 (water), 6.1 (M KCl), Corg 1.1%, WHC: 59.5 g/100 g dry soil.

Test design: Determination of carbon transformation in soil after addition of glucose (concentration in soil: 0.2%, w/w). Comparison of test item treated soil with a non-treated soil. 3 replicates per treatment and concentration were taken. A "gas handling unit and an infrared gas analyzer was used to measure the respiration and CO2 evolution over a period of ≥ 12 hours at different sampling intervals. Sampling scheme: 0, 14 and 28 days after treatment. Sub-samples were withdrawn from the bulk batches and subjected to measurement.

Endpoints: Effects on soil respiration via CO2 concentration measurements 0, 14 and 28 days after application.

Test concentrations: Control and 8.0 mg a.s./kg dry soil (equivalent to 6 kg BAS°610°F/ha); test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm3.

Reference item: Dinoseb (purity: 99.9%). The reference item was tested at concentrations of 1 and 10 mg/kg dry soil.

Test conditions: Water content: approx. 45% of maximum water holding capacity. Soil samples were incubated at 20 ± 2 °C while stored in plastic vessels in the dark.

Statistics: Descriptive statistics; Student's two sample t-test ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

No adverse effects of BAS 610 F on carbon transformation in soil could be observed in the test item concentration (8.0 mg a.s./kg dry soil) after 28 days. Only a negligible deviation from the control of +2.6 % was measured at the end of the 28-day incubation period. The results are summarized in Table 8.7-1.

Table 8.7-1: Effects of BAS 610 F on soil micro-organisms (carbon transformation) on days 0, 14 and 28 of incubation

Days	Control soil (with lucerne)	8.0 mg a.s./kg dry soil	
	Respiration [mL produced CO_2/100g soil/h]	Respiration [mL produced CO_2/100g soil/h]	% deviation from the control [1)]
sandy loam / loamy sand (0 d)	1.96	1.93	-1.5
sandy loam / loamy sand (14 d)	1.93	1.97	+2.1
sandy loam / loamy sand (28 d)	1.55	1.59	+2.6

1) - = inhibition; + = stimulation based on CO_2 production.

The reference item dinoseb caused an inhibition of carbon transformation of -0.6 %, and 28.5 % at 1 mg and 10 mg dinoseb/kg dry soil after 28 days of incubation, respectively. The latter value was significantly different from the control.

III. CONCLUSION

BAS 610 F (iprodione) caused no adverse effects (deviation from control < 25%, OECD 217) on the soil carbon transformation (measured as carbon dioxide production) at the end of the 28-day incubation period. The study was performed at a concentration of 8.0 mg a.s./kg dry soil.

Report:	CA 8.7/2 McMurray A., 1999a A laboratory assessment of the effects of RP 30228 on soil microflora respiration and nitrogen transformations according to current EU guidelines C022588
Guidelines:	EPPO Bulletin 24 (1994)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

In a soil microbial activity study, the effects of RP 30228 on the carbon transformation were investigated in a loamy sand soil and a sandy loam soil. RP 30228 was applied to samples of the soil at a test concentration of 6 kg/ha. Treated and untreated soils were incubated at approximately 20 ± 2 °C in the dark.

No adverse effects of RP 30228 on carbon transformation in soil could be observed in the test item concentration (6 kg RP 30228) after 28 days in the sandy loam soil and after 42 days in the loamy sand soil. Only a negligible deviation from the control of +1.11 % was measured at the end of the 28-day incubation period in the sandy loam soil. In the loamy sand soil significant effects were detected in comparison to the control after 28 days. Therefore the test was prolonged to 42 days. After test termination no significance was detected and the -11.90% effect in comparison to the control was determined.

RP 30228 (metabolite of iprodione, BAS 610 F) caused no adverse effects (deviation from control < 25%, OECD 217) on the soil carbon transformation (measured as carbon dioxide production) at the end of the 28-day incubation period. The study was performed at a concentration of 6 kg/ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: RP 30228 (metabolite of iprodione, BAS 610 F), batch no. DP653, purity 99.4% (w/w).

B. STUDY DESIGN

Test soil: Biologically active agricultural soils: a sandy loam (BBA classification): soil pH 7.3 (in water), Corg 1.5%, WHC 57.7 g/100 g dry soil;
a loamy sand (BBA classification): soil pH 5.9 (in water), Corg 2.7%, WHC 59.3 g/100 g dry soil

Test design: Determination of carbon transformation in soil after addition of glucose (concentration in soil: 0.2%, w/w). Comparison of test item treated soil with a non-treated soil. 3 replicates per treatment and concentration were taken. An infrared gas analyzer was used to measure the respiration and CO2 evolution overnight at different sampling intervals. Sampling scheme: 0, 14 and 28 days after treatment for the sandy loam and 0, 14, 28 and 42 days after treatment for the loamy sand. Sub-samples were withdrawn from the bulk batches and subjected to measurement.

Endpoints: Effects on soil respiration via CO2 concentration measurements 0, 14, 28 and 42 days after application.

Test concentrations: Control and 6 kg/ha (applied in quartz sand that was even distributed in the test soil).

Reference item: Dinoseb. The reference item was tested at a rate of 100 L/ha in a separate study-

Test conditions: Water content: approx. 45% of maximum water holding capacity. Soil samples were incubated at 20 ± 2 °C while stored in plastic vessels in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of RP 30228 on carbon transformation in soil could be observed in the test item concentration (6 kg RP 30228) after 28 days in the sandy loam soil and after 42 days in the loamy sand soil. Only a negligible deviation from the control of +1.11 % was measured at the end of the 28-day incubation period in the sandy loam soil. In the loamy sand soil significant effects <25% were detected in comparison to the control after 28 days ($\alpha = 0.05$). However, the test was prolonged to 42 days. After test termination no significance was detected and the -11.90% effect in comparison to the control was determined. The results are summarized in Table 8.7-2.

Table 8.7-2: Effects of RP 30228 on soil micro-organisms (carbon transformation) on days 0, 14, 28 and 42 of incubation

Days	Sandy loam soil			Loamy sand soil		
	Control soil (with lucerne)	6 kg/ha		Control soil (with lucerne)	6 kg/ha	
	Respiration [mL produced CO_2/100g soil/h]	Respiration [mL produced CO_2/100g soil/h]	% deviation from the control [1)]	Respiration [mL produced CO_2/100g soil/h]	Respiration [mL produced CO_2/100g soil/h]	% deviation from the control [1)]
0	2.02	2.13	+5.45	2.83	2.69	-4.95
14	1.69	1.81	+7.10	2.02	1.72	-14.85
28	1.80	1.82	+1.11	1.96	1.77 *	-9.69
42	not determined			1.68	1.48	-11.90

1) - = inhibition; + = stimulation based on CO_2 production.

* statistically significantly different from the control ($\alpha = 0.05$).

In a separate study the reference item dinoseb caused inhibitions of carbon transformation of 52.5% after 44 days in a sandy loam soil and of 55.06% after 28 days in a loamy sand soil, both at 100 L/ha. Both values were significantly different from the controls ($\alpha = 0.05$).

III. CONCLUSION

RP 30228 (metabolite of iprodione, BAS 610 F) caused no adverse effects (deviation from control < 25%, OECD 217) on the soil carbon transformation (measured as carbon dioxide production) at the end of the 28-day incubation period. The study was performed at a concentration of 6 kg/ha.

Report:	CA 8.7/3 McMurray A., 2001a A laboratory assessment of the effects of RP32596 (3,5-DCA) on soil microflora respiration and nitrogen transformations according to current EU-guidelines C014354
Guidelines:	EPPO Bulletin 24 (1994)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

In a soil microbial activity study, the effects of RP 32596 on the carbon transformation were investigated in a humus sandy soil and a sandy clay soil. RP 32596 was applied to samples of the soil at a test concentration of 6 kg/ha. Treated and untreated soils were incubated at approximately 20 ± 2 °C in the dark.

No adverse effects of RP 32596 on carbon transformation in soil could be observed in the test item concentration (6 kg RP 32596) after 28 days in a humus sandy soil and a sandy clay soil after 28 days. Statistically significant effects of +12.93% in the test item treatment compared to the control were found in the humus sandy soil after on day 0. However, after 14 and 28 days, only small deviations from the control of -0.75% and of -1.04% were measured in the humus sandy soil and in the sandy clay soil, respectively.

RP 32596 (metabolite of iprodione, BAS 610 F) caused no adverse effects (deviation from control < 25%, OECD 217) on the soil carbon transformation (measured as carbon dioxide production) at the end of the 28-day incubation period. The study was performed at a concentration of 6 kg/ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: RP 32596 (3,5-DCA) (metabolite of iprodione, BAS 610 F), batch no. 29027/8, purity 99.1% (w/w).

B. STUDY DESIGN

Test soil: Biologically active agricultural soils: a humus sandy soil and a sandy clay soil (BBA classification), soil characteristics:
humus sandy soil: pH 6.2 (in water), Corg 5.4%, WHC 30.17 g/100 g soil; sandy clay soil: pH 8.2 (in water), Corg 1.9%, WHC 20.50 g/100 g soil.

Test design: Determination of carbon transformation in soil after addition of glucose (concentration in soil: 0.2%, w/w). Comparison of test item treated soil with a non-treated soil. 4 replicates per treatment and concentration were taken. An infrared gas analyzer was used to measure the respiration and CO2 evolution overnight at different sampling intervals. Sampling scheme: 0, 14 and 28 days after treatment. Sub-samples were withdrawn from the bulk batches and subjected to measurement.

Endpoints: Effects on soil respiration via CO2 concentration measurements 0, 14 and 28 days after application.

Test concentrations: Control and 6 kg/ha (equivalent to 8.0 mg/kg dry soil, applied in quartz sand that was even distributed in the test soil).

Reference item: Dinoseb (purity: 99.9%). The reference item was tested at a rate of 56 kg/ha in a separate study.

Test conditions: Water content: approx. 55% of maximum water holding capacity. Soil samples were incubated at 20 ± 2 °C while stored in plastic vessels in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects RP 32596 on carbon transformation in soil could be observed in the test item concentration (6 kg RP 32596) after 28 days in a humus sandy soil and a sandy clay soil after 28 days. Statistically significant effects of +12.93% in the test item treatment compared to the control were found in the humus sandy soil after on day 0 ($\alpha = 0.05$). However, after 14 and 28 days, only small deviations from the control of -0.75% and of -1.04% were measured in the humus sandy soil and in the sandy clay soil, respectively. The results are summarized in Table 8.7-3.

Table 8.7-3: Effects of RP 32596 on soil micro-organisms (carbon transformation) on days 0, 14 and 28 of incubation

Days	Humus sandy soil			Sandy clay soil		
	Control soil (with lucerne)	6 kg/ha		Control soil (with lucerne)	6 kg/ha	
	Respiration [mL produced CO_2/100g soil/h]	Respiration [mL produced CO_2/100g soil/h]	% deviation from the control [1)]	Respiration [mL produced CO_2/100g soil/h]	Respiration [mL produced CO_2/100g soil/h]	% deviation from the control [1)]
0	1.16	1.31	+12.93 *	1.47	1.49	+1.36
14	1.48	1.49	+0.68	2.25	2.30	+2.22
28	1.33	1.32	-0.75	1.92	1.90	-1.04

1) - = inhibition; + = stimulation based on CO_2 production.

* statistically significantly different from the control ($\alpha = 0.05$).

In a separate study the reference item dinoseb caused inhibitions of carbon transformation of 59.9% after 28 days in a silty sand soil at 56 kg/ha.

III. CONCLUSION

RP 32596 (metabolite of iprodione, BAS 610 F) caused no adverse effects (deviation from control < 25%, OECD 217) on the soil carbon transformation (measured as carbon dioxide production) at the end of the 28-day incubation period. The study was performed at a concentration of 6 kg/ha.

Report:	CA 8.7/4 Witte B., 2010a Acute toxicity (14 days) of BAS 610 F (Iprodione) to the earthworm Eisenia fetida in artificial soil with 5% peat 2010/1075819
Guidelines:	OECD 207 (1984), ISO 11268-1 (1993)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

In an acute toxicity laboratory study adults of Eisenia fetida (Annelida: Oligochaeta) were exposed to BAS 610 F (iprodione). The test item was mixed into artificial soil (5 % peat only) at rates of 198, 296, 444, 667 and 1000 mg BAS 610 06 F/kg dry soil. For the control treatment, the soil was left untreated.

After 14 days of exposure, no mortality was observed in the test item concentrations and in the control. The biomass development was not statistically significantly different compared to the control up to and including 667 mg a.s./kg dry soil. At the highest concentration of 1000 mg a.s./kg dry soil, the body weight was statistically significantly reduced compared to the control. No behavioral effects were observed in any treatment group.

In a 14-day acute toxicity study to earthworms (Eisenia fetida) with BAS 610 F (iprodione) the LC50 was estimated to be > 1000 mg BAS 610 F/kg dry soil. The overall NOEC was determined to be 667 mg BAS 610 F/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 610 F; batch no. COD-000709; content of a.s.: iprodione (BAS 610 F, Reg. no. 101 169): 97.8 % (± 1.0 %).

B. STUDY DESIGN

Test species: Earthworm (Eisenia fetida), adult worms (with clitellum and weight 300 mg – 600 mg), age: 10-11 months old; source: in-house culture.

Test design: 14-day exposure in treated artificial soil with 5 % peat according to OECD 207. Different concentrations of the test item were mixed homogeneously into the soil which was then used to fill glass vessels after which the earthworms were introduced on top of the soil. 6 treatment groups were set up (5 test item concentrations, control); 4 replicates per treatment group with 10 worms each. Assessment of worm mortality and behavioral effects was carried out after 7 and 14 days, measurement of weight change as sub-lethal parameter after 14 days.

Endpoints: Mortality, behavioral effects, weight change.

Test concentrations: Control, 198, 296, 444, 667, 1000 mg BAS 610 F/kg dry soil.

Reference item: 2-chloroacetamide. The effects of the reference item were investigated in a separate study.

Test conditions: Artificial soil (5 % peat); pH 5.8 – 5.9 at test initiation, pH 5.6 – 5.7 at test termination; water content: 54.8 % – 56.3 % of water holding capacity (WHC) at test initiation, 52.8 % – 56.5 % of WHC at test termination; temperature: 18°C – 22°C; continuous illumination at 400-800 lux.

Statistics: Descriptive statistics, Dunnett's t-test for weight change ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

The LC_{50} was estimated to be > 1000 mg BAS 610 F /kg dry soil.

After 14 days of exposure, no mortality was observed in the test item concentrations and in the control. The biomass development was not statistically significantly different compared to the control up to and including 667 mg a.s./kg dry soil. At the highest concentration of 1000 mg a.s./kg dry soil, the body weight was statistically significantly reduced compared to the control (Dunnett-t-test, $\alpha = 0.05$). No behavioral effects were observed in any treatment group. The results are summarized in Table 8.7-4.

Table 8.7-4: Effects of BAS 610 F on earthworm (*Eisenia fetida)* mortality and biomass (14 days)

BAS 610 F [mg a.s./kg dry soil]	Control	198	296	444	667	1000
Mortality [%]	0	0	0	0	0	0
Weight change [%]	-6.9	-9.6	-8.0	-10.5	-12.3	-18.8 *
Endpoints [mg a.s./kg dry soil]						
LC_{50}	> 1000					
NOEC	667					

* = statistically significantly different to the control (Dunnett-t-test, $\alpha = 0.05$).

III. CONCLUSION

In a 14-day acute toxicity study to earthworms (*Eisenia fetida)* with BAS 610 F (iprodione) the LC_{50} was estimated to be > 1000 mg BAS 610 F/kg dry soil. The overall NOEC was determined to be 667 mg BAS 610 F/kg dry soil.

CA 8.8 Effects on biological methods for sewage treatment

Since Annex I inclusion of iprodione (BAS 610 F), new studies have been performed for investigation of the effects on biological methods for sewage treatment.

Report:	CA 8.8/1 Bachner H., 2004a BAS 610 F - Determination of the inhibition of oxygen consumption by activated sludge in the activated sludge respiration inhibition test 2004/1014970
Guidelines:	OECD 209, EEC 88/302, ISO 8192 - 1986 (E) (Method B)
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

The effect of iprodione (BAS 610 F) on the respiration rate of activated sludge collected from a wastewater treatment plant was determined according to OECD 209 and ISO 8192. Iprodione was tested at nominal concentrations of 10, 100, 250, 500 and 1000 mg/L. The O2 consumption rate of aerobic microorganisms was assessed after a contact period of 180 min under aerobic incubation at 20 ± 2 C.

No adverse effects of iprodione on aerobic microorganisms in activated sludge (measured as O2 consumption) could be observed after 180 minutes of incubation. Only negligible deviations from the control of +33% were measured at 1000 mg a.s./L.

The EC_{20}, EC_{50} and EC_{80} values of iprodione (BAS 610 F) in the activated sludge respiration inhibition test were ~650, > 1000 and > 1000 mg a.s./L, respectively. Disturbances in the bio-degradation process of activated sludge are not to be expected if the test item is correctly introduced into adapted wastewater treatment plants at low concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Iprodione (BAS°610°F, Reg. No. 101°169); charge no. FF 16009, purity: 97.8%.

B. STUDY DESIGN

Inoculum: Activated sludge from laboratory wastewater plant treating municipal sewage; inoculum: concentration of dry substance: 1 g/L.

Test design: 3 treatment groups: 5 different concentrations of the test item and 3 replicates of untreated control and reference item, respectively. The inoculum was washed, adjusted to 5 g/L dry substance and aerated overnight. 50 mL were added to a total volume of 250 mL, resulting in a test concentration of 1 g dry substance/L. O2 concentration during aeration: > 2.5 mg/L, O2 concentration immediately before measurement: > 6.5 mg/L. Duration of the measurement of O2 consumption: 8-10 min. Comparison of O2 consumption of the test substance and an untreated control after 180 min of incubation.

Endpoints: Effects on the O2-consumption after 180 min of incubation.

Test concentrations: Untreated control; test item treatment: 10, 100, 250, 500 and 1000 mg a.s./L (nominal).

Test conditions: Incubation at 20 ± 2°C while stirred in 250 mL Erlenmeyer vessels for 180 min.

II. RESULTS AND DISCUSSION

No adverse effects of iprodione on aerobic microorganisms in activated sludge (measured as O_2 consumption) could be observed after 180 minutes of incubation. Only negligible deviations from the control of +33% were measured at 1000 mg a.s./L. The results are summarized below in Table 8.8-1.

Table 8.8-1: Effects of iprodione (BAS°610°F) on inhibition of O_2 consumption in activated sewage sludge after 180 minutes

Concentration [mg a.s./L] (nominal)	**Control**	**10**	**100**	**250**	**500**	**1000**
O_2 consumption rate [mg O_2/L*h]	15	16	16	13	13	10
% deviation from control	--	-7	-7	13	13	33
	Endpoints [mg a.s./L] (nominal)					
EC_{20}	~ 650					
EC_{50}	> 1000					
EC_{80}	> 1000					

Negative values indicate an increase of O_2 consumption.

III. CONCLUSION

The EC_{20}, EC_{50} and EC_{80} values of iprodione (BAS 610 F) in the activated sludge respiration inhibition test were ~650, > 1000 and > 1000 mg a.s./L, respectively. Disturbances in the bio-degradation process of activated sludge are not to be expected if the test item is correctly introduced into adapted wastewater treatment plants at low concentrations.

Report:	CA 8.8/2 Watt S.D., 1994a Iprodione - Acute toxicity in bacteria (Pseudomonas putida) R000325
Guidelines:	none
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

In a bacterial growth test, the acute toxicity of iprodione (BAS°610°F) on *Pseudomonas putida* was investigated. Three identical dilution series of the test item were inoculated with *P. putida,* resulting in three test cultures at 2-fold concentrations ranging from 0.005 to 10.4 mg a.s./L, respectively. An untreated control was included in the test. The cultures were incubated at 25 °C (± 1 °C) for 16 h (± 1 h).

No adverse effects of iprodione on *P. putida* were observed at the end of the cultivation period after 16 hours. Deviations from the control ranged from -1.7% at 0.005 mg a.s./L to 9.6% at 10.4 mg a.s./L, respectively. Due to the absence of adverse effects, no EC_{10} or EC_{50} value could be determined

In a bacterial growth test, iprodione (BAS 610 F) did not inhibit the growth of *Pseudomonas putida* at its maximum level of solubility and thus, the EC_{10} and EC_{50} values of iprodione could not be determined.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Iprodione (BAS°610°F, Reg. No. 101°169); Lot no. EA2002SD9; CAS No. 36734-19-7; purity: 997 g/kg

B. STUDY DESIGN

Test species: Bacteria, Pseudomonas putida migula strain (designation Berlin 33/2 strain number DMS 50026 NCIMB 12708); harvested from a 1 - 7 day old stock culture.

Test design: 2 treatment groups: 12 different concentrations of the test item (3 replicates each) and 10 replicates of untreated control. The cultures were incubated at 25°C (± 1 h) for 16 hours (± 1 h). At the end of the incubation period, the extinction at 436 nm of the test and control cultures was determined using a Pye Unicam PU8600 UV/VIS Spectrophotometer.

Endpoints: EC10, EC50.

Test concentrations: Untreated control; twelve concentrations ranging from 0.005 to 10.4 mg a.s./L (factor between two consecutive concentrations: 2).

Test conditions: Incubation at 25°C ± 1°C for 16 hours (± 1 h).
t

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of iprodione on *P. putida* were observed at the end of the cultivation period after 16 hours. Deviations from the control ranged from -1.7% at 0.005 mg a.s./L to 9.6% at 10.4 mg a.s./L, respectively. Due to the absence of adverse effects, no EC_{10} or EC_{50} value could be determined. The results are summarized below in Table 8.8-1.

Table 8.8-2: Effects of iprodione (BAS°610°F) on bacterial growth of *Pseudomonas putida* after 16 h (± 1h)

Concentration [mg a.s./L] (nominal)	**Control**	**0.005**	**0.010**	**0.020**	**0.041**	**0.081**	**0.163**	**0.325**	**0.65**	**1.3**	**2.6**	**5.2**	**10.4**
Extinction [436 nm] [1)]	0.600	0.610	0.599	0.606	0.587	0.594	0.606	0.578	0.586	0.590	0.578	0.552	0.543
% deviation from control	--	-1.7	0.2	-1.0	2.1	1.0	-1.0	3.7	2.4	1.7	3.7	8.1	9.6
	Endpoints [mg a.s./L] (nominal)												
EC_{10}	n.d.												
EC_{50}	n.d.												

[1)] Mean value from 3 replicates.

III. CONCLUSION

In a bacterial growth test, iprodione (BAS 610 F) did not inhibit the growth of *Pseudomonas putida* at its maximum level of solubility and thus, the EC_{10} and EC_{50} values of iprodione could not be determined.

CA 8.9 Monitoring data

No monitoring studies assessing ecotoxicological effects of iprodione are available.

Iprodione

DOCUMENT M-CA, Section 9

LITERATURE DATA

Compiled by:

[redacted]

[redacted]

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Fax.No.:
E-mail:

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 Iprodione and the common product trade names was performed by the BASF Group Information Center. The Literature Search Report on Iprodione 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 2014/1097869).

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; whereas the “hits” were further evaluated by the scientific experts and categorized into “relevant” (yes/no), “reliable” (yes/no), and “used for dossier”. This is documented in nine EXCEL files which are attached to the search report in K-CA 9 with the file names and results as listed in table below:

Iprodione Literature analytics_AIR III	No literature was identified to be of interest for use in the dossier
Iprodione Literature Ecotox aquatic_AIR III	1 hit was identified to be discussed in the dossier
Iprodione Literature Ecotox general_AIR III	No literature was identified to be of interest for use in the dossier
Iprodione Literature Ecotox terrestric_AIR III	1 hit was identified to be discussed in the dossier
Iprodione Literature Ecotox wildlife_AIR III	No literature was identified to be of interest for use in the dossier
Iprodione Literature E-fate_AIR III	8 hits were identified to be discussed in the dossier
Iprodione Literature Toxicology_AIR III	26 hits were identified to be discussed in the dossier
Iprodione Literature_metabolism animal_AIR III	No literature was identified to be of interest for use in the dossier
Iprodione Literature_metabolism residue plant_AIR III	2 hits were identified to be discussed in the dossier

In addition, a search was conducted on institutional websites. Following documentation from JMPR, US EPA and CA EPA have been retrieved and are provided in K-CA 9:

Iprodione JMPR Evaluation (1992)
(BASF DocID 1992/1005257)

Iprodione JMPR Evaluation (1994)
(BASF DocID 1994/1005369)

Iprodione JMPR Report (1994)
(BASF DocID 1994/1005370)

Iprodione JMPR Report (1995)
(BASF DocID 1995/1008094)

Iprodione JMPR Evaluation (2001)
(BASF DocID 2001/1031883)

Iprodione JMPR Report (2001)
(BASF DocID 2001/1031884)

Iprodione US EPA Pesticide Tolerance (1996)
(BASF DocID 1996/1006240)

Iprodione US EPA RED (1998)
(BASF DocID 1998/1009515)

Iprodione US EPA RED Facts (1998)
(BASF DocID 1998/1009516)

Iprodione US EPA Pesticide Tolerance (1999)
(BASF DocID 1999/1013797)

Iprodione US EPA Ecological Evaluation (RA for frogs) (2009)
(BASF DocID 2009/1130742)

Iprodione US EPA Dietary Exposure and RA (2012)
(BASF DocID 2012/1366202)

Iprodione US EPA Refined drinking water assessment for label amendment (2012)
(BASF DocID 2012/13662264)

Iprodione US EPA Registration review Preliminary problem formulation for ecological risk and environmental fate, endangered species, and drinking water assesments (2012)
(BASF DocID 2012/1366203)

Iprodione US EPA Human Health RA - Scoping Document (2012)
(BASF DocID 2012/1366204)

Iprodione US EPA Occupational Exposure and RA (2012)
(BASF DocID 2012/1366208)

Iprodione US EPA Preliminary Work Plan – Registration Review : Initial Docket, Case #2335 (December 2012)
(BASF DocID 2012/1366209)

Iprodione US EPA Final Work Plan – Registration Review Case #2335 (June 2013)
(BASF DocID 2013/1414723)

Iprodione California EPA - Summary of Tox Data (1986)
(BASF DocID 1986/1002751)

Iprodione

DOCUMENT M-CA, Section 10

CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

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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

Legislation	Directive 67/548/EEC	Regulation (EC) No 1272/2008
Classification	Possible Hazards: Limited evidence of a carcinogenic effect Category 3: Substances which cause concern for man due to possible carcinogenic effects, however, since sufficient information is not available a satisfactory assessment cannot be made. Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.	Carcinogenicity (Category 2) Acute aquatic toxicity (Category 1) Chronic aquatic toxicity (Category 1)
Labelling	**Hazard symbol(s) :** Xn, N  **R-phrase(s)** R40 : Limited evidence of a carcinogenic effect R50/53 :Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment **S-phrase(s)** S36/37 : Wear suitable protective clothing and gloves. S46: If swallowed, seek medical advice immediately and show this container or label. S60: This material and its container must be disposed of as hazardous waste. S61: Avoid release to the environment. Refer to special instructions/safety data sheets.	**Signal Word :** Warning  **Hazard Statement** H351 : Suspected of causing cancer. H400 : Very toxic to aquatic life H410 :Very toxic to aquatic life with long lasting effects **Precautionary Statements (Prevention):** P280 Wear protective gloves/protective clothing/eye protection/face protection. P273 Avoid release to the environment. P202 Do not handle until all safety precautions have been read and understood. **Precautionary Statements (Response):** P308 + P311 IF exposed or concerned: Cali a POISON CENTER or doctor/physician. P391 Collect spillage. Precautionary Statements (Storage): P405 Store locked up. **Precautionary Statements (Disposal):** P501 Dispose of contents/container to hazardous or special waste collection point.
Concentration limits	None	None