Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

dimoxystrobin (ISO); (2*E*)-2-{2-[(2,5-dimethylphenoxy)methyl]phenyl}-2-(methoxyimino)-*N*-methylacetamide; (*E*)-2-(methoxyimino)-*N*-methyl-2-[*a*-(2,5-xylyloxy)-o-tolyl]acetamide

EC Number: -
CAS Number: 149961-52-4

CLH-O-0000006865-62-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

8 October 2020

CLH Report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

Substance Name: Dimoxystrobin

EC Number: -

CAS Number: **149961-52-4**

Index Number: **616-164-00-7**

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Version number: 1 **Date: May 2019**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DIMOXYSTROBIN (ISO); (2*E*)-2-{2-[(2,5-DIMETHYLPHENOXY)METHYL]PHENYL}-2-(METHOXYIMINO)-*N*-METHYLACETAMIDE; (*E*)-2-(METHOXYIMINO)-*N*-METHYL-2-[α-(2,5-XYLYLOXY)-*o*-TOLYL]ACETAMIDE

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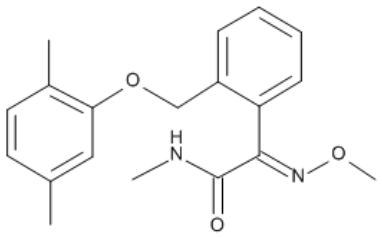
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	CAS name: Benzeneacetamide, 2-[(2,5-dimethylphenoxy)methyl]-α-(methoxyimino)-N-methyl-, (αE)- IUPAC name: (E)-o-(2,5-dimethylphenoxymethyl)-2-methoxyimino-N-methylphenylacetamide
Other names (usual name, trade name, abbreviation)	BAS 505 F
ISO common name (if available and appropriate)	Dimoxystrobin (ISO)
EC number (if available and appropriate)	-
EC name (if available and appropriate)	not assigned
CAS number	149961-52-4
Other identity code (if available)	-
Molecular formula	$C_{19}H_{22}N_2O_3$
Structural formula	
SMILES notation (if available)	CNC(=O)C(=NOC)c1ccccc1COc2cc(C)ccc2C
Molecular weight or molecular weight range	326.394 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	Minimum purity: 980 g/kg

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
Dimoxystrobin (ISO) CAS No. 149961-52-4	Not applicable	Carc. 2, H351 Repr. 2, H361d*** Acute Tox. 4*, H332 Aquatic Acute 1, H400 Aquatic Chronic 1, H410	Carc. 2, H351 Repr. 2, H361d*** Acute Tox. 4, H332 Aquatic Acute 1, H400 Aquatic Chronic 1, H410

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 3: Existing and proposed harmonised classification and labelling for dimoxystrobin according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	616-164-00-7	dimoxystrobin (ISO); (2E)-2-{2-[(2,5-dimethylphenoxy)methyl]phenyl}-2-(methoxyimino)-N-methylacetamide; (E)-2-(methoxyimino)-N-methyl-2-[α-(2,5-xylyloxy)-o-tolyl]acetamide	-	149961-52-4	Carc. 2 Repr. 2 Acute Tox. 4* Aquatic Acute 1 Aquatic Chronic 1	H351 H361d*** H332 H400 H410	GHS07 GHS09 GHS08 Wng	H351 H361d*** H332 H410			
Dossier submitter's proposal	616-164-00-7	dimoxystrobin (ISO); (2E)-2-{2-[(2,5-dimethylphenoxy)methyl]phenyl}-2-(methoxyimino)-N-methylacetamide; (E)-2-(methoxyimino)-N-methyl-2-[α-(2,5-xylyloxy)-o-tolyl]acetamide	-	149961-52-4	**Remove:** Repr. 2 **Add:** STOT RE 2 Lact. **Modify:** Acute Tox. 4	**Remove:** H361d*** **Add:** H373 (blood) H362	**Maintain**: GHS07 GHS09 GHS08 Wng	**Remove:** H361d*** **Add:** H373 (blood) H362		**Add:** Inhalation: ATE=1.3 mg/L (dust and mist) M = 100 M = 100	
Resulting Annex VI entry if agreed by RAC and COM	616-164-00-7	dimoxystrobin (ISO); (2*E*)-2-{2-[(2,5-dimethylphenoxy)methyl]phenyl}-2-(methoxyimino)-*N*-methylacetamide; (*E*)-2-(methoxyimino)-*N*-methyl-2-[α-(2,5-xylyloxy)-*o*-tolyl]acetamide	-	149961-52-4	Carc. 2 Lact. Acute Tox. 4 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H362 H332 H373 (blood) H400 H410	GHS07 GHS09 GHS08 Wng	H351 H362 H332 H373 (blood) H410		inhalation: ATE = 1.3 mg/L (dust and mist) M = 100 M = 100	

Table 4: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Data conclusive but not sufficient for classification	No
Flammable gases (including chemically unstable gases)	Data conclusive but not sufficient for classification	No
Oxidising gases	Data conclusive but not sufficient for classification	No
Gases under pressure	Data conclusive but not sufficient for classification	No
Flammable liquids	Data conclusive but not sufficient for classification	No
Flammable solids	Data conclusive but not sufficient for classification	No
Self-reactive substances	Data conclusive but not sufficient for classification	No
Pyrophoric liquids	Data conclusive but not sufficient for classification	No
Pyrophoric solids	Data conclusive but not sufficient for classification	No
Self-heating substances	Data conclusive but not sufficient for classification	No
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	No
Oxidising liquids	Data conclusive but not sufficient for classification	No
Oxidising solids	Data conclusive but not sufficient for classification	No
Organic peroxides	Data conclusive but not sufficient for classification	No
Corrosive to metals	Data conclusive but not sufficient for classification	No
Acute toxicity via oral route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Current classification: Acute Tox. 4*, H332 Harmful if inhaled. New classification proposed: Acute Tox. 4, H332 Harmful if inhaled.	Yes
Skin corrosion/irritation	Data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	No data available	No
Skin sensitisation	Data conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	Not assessed in this CLH dossier.	No
Carcinogenicity	Current classification: Carc. 2, H351 Suspected of causing cancer.	No
Reproductive toxicity	Current classification: Repr. 2, H361d*** Suspected of damaging the unborn child. New classification proposed: Effect on or via lactation, H362 May cause harm to breast-fed children.	Yes
Specific target organ toxicity-single exposure	Not assessed in this CLH dossier.	No
Specific target organ toxicity-repeated exposure	New classification proposed: STOT RE H373 May cause damage to organs (blood)	Yes
Aspiration hazard	Data conclusive but not sufficient for classification	No

Hazard class	**Reason for no classification**	**Within the scope of public consultation**
Hazardous to the aquatic environment	Current classification: Aquatic Acute 1, H400 Very toxic to aquatic life. Aquatic Chronic 1, H410 Very toxic to aquatic life with long lasting effects. M-factor 10 New classification proposed: Aquatic Acute 1, H400: Very toxic to aquatic life. Aquatic Chronic 1, H410: Very toxic to aquatic life with long lasting effects. M-factor 100 (acute and chronic)	Yes
Hazardous to the ozone layer	Data conclusive but not sufficient for classification	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Dimoxystrobin is an existing active substance for plant protection products and has been reviewed in accordance to Council Directive 91/414/EEC with The United Kingdom (UK) as Rapporteur Member State (RMS). It was included into Annex I with Commission Directive 2006/75/EC of 11 September 2006. The current harmonized classification (2005) is Acute Tox. Cat. 4* (inhalation), H332; Carc. Cat. 2, H351; Repro. Cat. 2, H361d***, Aquatic Acute 1, H400 and Aquatic Chronic 1, H410 with an M-factor of 10.

During the review process of dimoxystrobin as an active substance for plant protection products, no classification for reproductive toxicity was proposed by either the RMS UK or EFSA as "There were no direct effects on reproductive performance or fertility observed up to the highest dose level. […] Dimoxystrobin did not induce teratogenic or fetotoxic effects."

Dimoxystrobin was discussed at the European Chemicals Bureau (ECB) in Ispra. In March, 2005 at the ECB level, classification as Xn, R20 for acute inhalation toxicity and Carc. Cat. 3, R40 were agreed. A single member state sent in a proposal to classify as Repr. Cat.3, R63. It was pointed out, that the proposal did not include any data not already discussed at the March meeting and that the Technical Committee for Classification and Labelling (TC C&L) had already agreed on no classification based on this data. In November 2005 TC C&L agreed with the proposal to classify as Repr. Cat. 3, R63. There is no officially documented rationale for the classification of dimoxystrobin as Repr. Cat. 3, R63 (Repr. Cat. 2, H361d*** according to the CLP Regulation).
From the available meeting documents, however, it can be assumed that the observed effects on body weights, hearts (cardiomegaly) and blood (anemia) in the offspring of the reproduction toxicity studies constituted the basis for the agreed decision to classify as R63. Further, a higher susceptibility of the offspring compared to the adults was assumed.

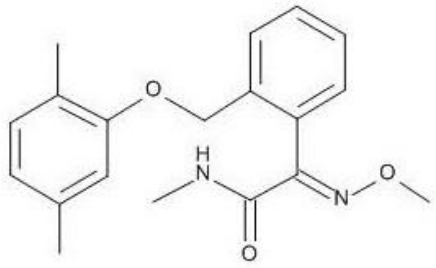
Since then, new mechanistic data on reproductive toxicity of dimoxystrobin and the assumed susceptibility of young vs. older rats were generated. These new data have been submitted according to Regulations (EC) 1107/2009 and 844/2012 within the supplementary dossier for the renewal of approval of dimoxystrobin as active substance for plant protection products to RMS Hungary (HU) and co-RMS Ireland (IE). The data are presented in the context of the results of the generation toxicity studies and the mechanistic investigations. The present CLH report concludes that the classification with Repr. Cat. 2 is not justified. Instead, a classification for lactation, H362 seems to be appropriate. Additionally, the developmental toxicity studies conducted in rats and rabbits are also summarized in this CLH report. Finally, a comparison with the CLP criteria is made.

For acute toxicity, the classification of dimoxystrobin is considered as minimum classification as it was based on the criteria of Directive 67/548/EEC which do not correspond directly to the classification in a

hazard class and category of Regulation (EC) No 1272/2008. Detailed summaries for the acute toxicity studies are presented and a comparison with the CLP criteria is made.

RAC general comment

Dimoxystrobin is an existing fungicidal active substance for use in plant protection products. It is mainly used to control a range of fungal diseases on oilseed rape and sunflower. The chemical structure of dimoxystrobin is shown in the Figure below:



Dimoxystrobin has a current harmonized classification as Acute Tox. Cat. 4* (inhalation), H332; Carc. Cat. 2, H351; Repro. Cat. 2, H361d***, Aquatic Acute 1, H400 and Aquatic Chronic 1, H410 with an M-factor of 10; although there is no officially documented rationale for such classification.

Since the original harmonised classification of dimoxystrobin, new mechanistic data on reproductive toxicity were generated. These new data have been submitted according to Regulations (EC) 1107/2009 and 844/2012 within the supplementary dossier for the renewal of approval of dimoxystrobin as active substance in plant protection products. The dossier submitter (DS) concludes in the CLH-report that the classification with Repr. Cat. 2 is not justified. Instead, a classification for effects on or via lactation, H362 as well as STOT RE 2, H373 (blood) was proposed.

During the consultation, one member state competent authority (MSCA) requested clarification about whether the active substance contains relevant impurities. The DS replied that dimoxystrobin does not contain toxicologically relevant impurities at the specified limit.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Specific justification that action is needed at EU level is not required, since dimoxystrobin is an active substance for plant protection products under Regulation (EC) 1107/2009.

5 IDENTIFIED USES

Dimoxystrobin is an existing fungicidal active substance for use in plant protection products. It is mainly used to control a range of fungal diseases on oilseed rape and sunflower.

6 DATA SOURCES

Dimoxystrobin is an approved active substance for plant protection products under Regulation (EC) 1107/2009 and has been placed on the market in the EU since 2003. The present evaluation relies on data submitted in the context of the former application for inclusion into Annex I to Directive 91/414/EEC and the currently ongoing evaluation for the renewal of approval as an active substance under Regulations (EC) 1107/2009 and 844/2012.

7 PHYSICOCHEMICAL PROPERTIES

Table 5: Summary of physicochemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Physical state at 20 °C and 101,3 kPa	PAI (99.9 %): white crystalline solid; TGAI (97.9 %): beige fine powder	EFSA Scientific Report (2005) 45, 1-82	Information previously reported and peer-reviewed
Melting/freezing point	PAI (99.9 %): Melting range: 138.1 – 139.7 °C; Decomposition temperature: 300 °C	EFSA Scientific Report (2005) 45, 1-82	Information previously reported and peer-reviewed
Boiling point	PAI (99.9 %): No boiling point up to decomposition at 300 °C	EFSA Scientific Report (2005) 45, 1-82	Information previously reported and peer-reviewed
Relative density	Relative density, PAI (99.9 %): D_4^{20} = 1.235 (20 °C)	EFSA Scientific Report (2005) 45, 1-82	Information previously reported and peer-reviewed
Vapour pressure	Vapour pressure, PAI (99.9 %) : $p = 6.0 \cdot 10^{-9}$ hPa (20 °C) $p = 1.4 \cdot 10^{-8}$ hPa (25 °C)	Kaestel, 1997a	Measured
	Henry´s Law Constant: $H = 5.9 \cdot 10^{-5}$ Pa · m^3 · mol^{-1}	Kroehl, 2015a	Calculated
Surface tension	Surface tension, TGAI (97.9 %): 64.3 mN/m at 20 °C and 0.5% w/w in water, 63.0 mN/m at 20 °C and 1.0% w/w in water	EFSA Scientific Report (2005) 45, 1-82	Information previously reported and peer-reviewed
	Surface tension, PAI (99.9 %): 66.3 mN/m at 20 °C and 0.5% w/w in water, 65.2 mN/m at 20 °C and 2.0% w/w in water	Kaestel, 1997a	Measured
Water solubility	Results were determined applying the column elution method, PAI (99.9 %): pH 5.7: 4.3 mg/L at 20 °C pH 8.0: 3.5 mg/L at 20 °C	EFSA Scientific Report (2005) 45, 1-82	Information previously reported and peer-reviewed
	Results were determined applying the column elution method, PAI (99.9 %): pH 5.0: 3.324 mg/L at 20 ± 0.4 °C	Fieseler, 2014a	Measured
Partition coefficient n-octanol/water	PAI (99.9 %): pH 6.5: log P_{OW} = 3.59 at 22 °C	EFSA Scientific Report (2005) 45, 1-82	Information previously reported and peer-reviewed
Flash point	Not applicable (melting point > 40 °C).	-	-

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Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Flammability	Flammability: not flammable.	EFSA Scientific Report (2005) 46, 1-82	Information previously reported and peer-reviewed
Explosive properties	No potential for explosivity as evident from the structural formula and the calculated oxygen balance (-224)	EFSA Scientific Report (2005) 46, 1-82	Information previously reported and peer-reviewed
	Pre-test via DSC: 1st reaction: onset 170°C, peak 296°C, energy release 470 J/g (exothermal) 2nd reaction: onset 390°C, energy release > 70 J/g (exothermal) Testing on explosive properties (thermal and mechanical sensitivity and friction): negative Conclusion: The test substance is not considered to exhibit a danger of explosion.	Achhammer G., 2013a	Measured
Self-ignition temperature	Auto flammability: no self-heating at temperatures up to the melting point.	EFSA Scientific Report (2005) 46, 1-82	Information previously reported and peer reviewed
Oxidising properties	Not oxidizing.	EFSA Scientific Report (2005) 46, 1-82	Information previously reported and peer reviewed
Granulometry	N/A	-	-
Solubility in organic solvents	PAI (99.7 %) at 20 °C: dichloromethane > 250 g/L DMF 200 – 250 g/L acetone 67 – 80 g/L acetonitrile 50 – 57 g/L ethyl acetate 33 – 40 g/L toluene & methanol 20 – 25 g/L 2-propanol < 10 g/L n-heptane < 10 g/L 1-octanol < 10 g/L olive oil < 10 g/L	EFSA Scientific Report (2005) 45, 1-82	Information previously reported and peer-reviewed
	Results (mass per volume of solution) were obtained at 20 ± 1 °C applying the shake flask method, TGAI (97.4 %): n-heptane 216.8 mg/L p-xylene 12.98 g/L dichloromethane > 250 g/L methanol 21.71 g/L acetone 73.42 g/L acetonitrile 57.06 g/L ethyl acetate 44.32 g/L	Fieseler, 2014b	Measured
Dissociation constant	No indication of dissociation of dimoxystrobin in water.	EFSA Scientific Report (2005) 45, 1-82	Information previously reported and peer-reviewed
Viscosity	At a shear rate of 100 s^{-1} at 20 °C : 90.7 mPas. The sample shows plastic behaviour.	-	Kaestel, 2001

8 EVALUATION OF PHYSICAL HAZARDS

Not relevant as no changes to the existing harmonized classification are proposed.

No classification is triggered by the physical/chemical properties.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Dimoxystrobin is rapidly absorbed. Concerning the bioavailability the RMS stated to prefer to calculate oral absorption based on measured data for urine and bile rather than on extrapolated data, so the RMS considered a mean bioavailability of 80%, when rats were exposed to the low dose level (10 mg/kg bw) and 58% when rats were exposed to the high dose level (100 mg/kg bw)**.** The applicant reported a bioavailability in the range of 85 - 90% at the low dose level (10 mg/mg bw) and 58 - 71% at the high dose level (100 mg/kg bw).
Dimoxystrobin is rapidly excreted via urine and feces. The majority of the radioactivity was excreted via feces (57 - 82% of the dose) and smaller amounts via urine (14 - 39% of the dose). The renal excretion was more pronounced in female animals. There is very little evidence of any cumulative potential of dimoxystrobin. Throughout the time course of the experiments, highest radioactivity concentrations were found in the gastrointestinal tract. Tissues and organs which showed radioactivity concentrations higher than or close to the plasma concentration were kidney, liver, lung, fat tissue, thyroid, pancreas, adrenals, ovaries, uterus.
Dimoxystrobin is extensively metabolised. In total 3 major transformation steps were observed in rats:

- hydroxylation of the aromatic ring system and oxidation of the methyl groups on the phenyl moiety
- cleavage of the ether bond between the ring systems
- side chain modifications (demethylation reactions, oxidation reactions)

The combination of these reactions followed by conjugation steps results in a huge number of metabolites.
The metabolite patterns in feces, urine, bile, liver, kidney and plasma were largely comparable for both sexes and for all dose groups investigated.
No human-specifics metabolites were found.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

For acute toxicity, the classification of dimoxystrobin is considered as minimum classification as it was based on the criteria of Directive 67/548/EEC which do not correspond directly to the classification in a hazard class and category of Regulation (EC) No 1272/2008. Detailed summaries for the acute toxicity studies are presented and a comparison with the CLP criteria is made.

10.1 Acute toxicity – oral route

Table 6: Summary of table of animal study on acute oral toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, purity, vehicle**	**Dose levels, vehicle, duration of exposure**	**Value** **LD_{50}**	**Reference**
Acute toxicity, oral, Equivalent to OECD 401 (1987) GLP compliant	Rat, Wistar, male and female, 5/sex in 2 subgroups	Dimoxystrobin (N6 Lot 3004) Purity: 98.8% Vehicle: 0.5 % Tylose	2000, 5000 mg/kg bw, single oral administration (gavage) 14-days post dose observation	LD_{50} > 5000 mg/kg bw	Anonymous, 1998 1998/11002

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

There was no mortality. Signs of toxicity noted at 2,000 and 5,000 mg/kg bw included impaired or poor general state, dyspnoea, apathy, staggering, and diarrhoea in males and females. All animals appeared normal within six days after application. Body weight gain generally appeared to be normal. There were no macroscopic pathological findings in animals sacrificed at the end of the observation period.

The oral LD_{50} of dimoxystrobin was > 5,000 mg/kg bw for male and female rats.

10.1.2 Comparison with the CLP criteria

The oral LD50 of > 5000 mg/kg bw for rats is above the value for classification provided in the Regulation. No classification for acute oral toxicity.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Not classified – Conclusive but not sufficient for classification

10.2 Acute toxicity - dermal route

Table 7: Summary of table of animal study on acute dermal toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, purity, vehicle**	**Dose levels duration of exposure**	**Value LD_{50}**	**Reference**
Acute toxicity, dermal, Equivalent to OECD 402 (1987) GLP compliant	Rat, Wistar, male and female, 5/sex	Dimoxystrobin (N6 Lot 3004) Purity: 98.8% Vehicle: 0.5 % Tylose	2000 mg/kg bw, single dose under a semiocclusive dressing, 24 h	LD_{50} > 2000 mg/kg bw	Anonymous, 1998 1998/11001

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

No mortality occurred and no clinical signs of toxicity were observed. No effects on the skin were observed (recorded 30-60 minutes after removal of dressing and weekly thereafter). Body weight development appeared to be normal with exception of one female animal, which showed weight reduction and another female animal, which showed no gain in body weight, in the first week of observation. No pathological findings were detected in the animals.

The dermal LD_{50} of dimoxystrobin was > 2000 mg/kg bw.

10.2.2 Comparison with CLP criteria

The dermal LD50 of >2000 mg/kg bw for rats is above the value for classification provided in the Regulation. No classification for acute dermal toxicity is proposed.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Not classified – Conclusive but not sufficient for classification.

10.3 Acute toxicity - inhalation route

Table 8: Summary of table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD), vehicle**	**Dose levels, duration of exposure**	**Value** LC_{50}	**Reference**
Acute toxicity, inhalation, Equivalent to OECD 403 (1981) GLP compliant	Rat, Wistar, male and female, 5/sex/dose group	Dimoxystrobin (N6 Lot 3004) Purity: 98.8% dust aerosol MMAD (low and mid dose): 2.5 µm MMAD (high dose): 5.1 µm Vehicle: none	0.51, 1.28, 5.9 mg/L single head-nose inhalation (4-hour exposure)	LC_{50} 1.9 mg/l (males), 1.3 mg/l (females) (4h)	Anonymous, 1997, 1998 Acute inhalation toxicity in Wistar rats

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

All animals exposed to 5.9 mg/L died during exposure. In the intermediate concentration of 1.28 mg/L one male and two female animals died during or apparently immediately after exposure. In the low and mid dose concentration group clinical examination revealed attempts to escape, irregular accelerated and intermittent respiration, as well as squatting posture and piloerection. No clinical signs could be detected from post exposure day 5 onward. The mid concentration resulted additionally in smeared fur. In the surviving animals no clinical signs were detected from day 8 onward. Necropsy of the decedent mid (1.28 mg/L) concentration animals showed agonal congestive hyperaemia. No macroscopic pathologic findings were noted in all other animals that died or were examined at the end of the study.

The inhalation LC_{50} of dimoxystrobin was in females 1.3 mg/L and in males 1.9 mg/L. The LC_{50} of both sexes combined is about 1.7 mg/L.

10.3.2 Comparison with CLP criteria

The CLP classification criteria for dusts and mists require a classification into Category 4, if LC_{50} values between 1.0 and 5.0 are determined. In addition, in agreement with the CLP Regulation (section 3.1.2.1 and Table 3.1.1), the acute toxicity estimate (ATE) for the classification of a mixture is derived using the LC_{50}. Since the lowest LC_{50} is 1.3 mg/L for female rats, the ATE for dimoxystrobin (dust and mist) by inhalation can be set at 1.3 mg/L. Thus, based on the available study, dimoxystrobin is to be classified into Acute Tox. 4; H332.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Classification proposed as Acute Tox. 4; H332 (Harmful if inhaled) with an ATE for the classification of mixtures (inhalation) of 1.3 mg/L.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

DS proposed no classification for acute oral toxicity based on an OECD TG 401 and GLP compliant test showing a LD_{50} higher than 5000 mg/kg bw. DS proposed no classification for acute dermal toxicity based on an OECD TG 402 and GLP compliant test showing a LD_{50} higher than 2000 mg/kg bw. DS proposed the classification of dimoxystrobin as Acute Tox. 4; H332 based on an OECD TG 403 and GLP compliant test showing a LC_{50} of 1.9 mg/L for males and 1.3 mg/L for females, with an ATE value of 1.3 mg/L (dusts or mists).

Comments received during consultation

One MSCA supported the proposal of classification as Acute Tox. 4; H332.

A manufacturer company provided a more detailed summary of the acute toxicity studies that had also been provided to EFSA during the pesticide peer review process.

Assessment and comparison with the classification criteria

The table below summarises the available studies for acute toxicity of dimoxystrobin.

***Table**: Summary of animal studies on acute toxicity with dimoxystrobin.*

Study	Dose level	Results	Reference
Acute oral toxicity Equivalent to OECD TG 401 (1987) GLP compliant Wistar rats 5 animals/sex N6 Lot 3004	Purity: 98.8% Vehicle: 0.5 % tylose 2000, 5000 mg/kg bw Single oral administration (gavage) 14-days post dose observation	No mortalities Clinical signs (both doses): impaired or poor general state, dyspnoea, apathy, staggering, and diarrhoea All animals appeared normal within six days after application No macroscopic pathological findings **LD_{50} > 5000 mg/kg bw**	Anonymous, 1998 1998/11002
Acute dermal toxicity Equivalent to OECD TG 402 (1987) GLP compliant Wistar rats 5 animals/sex N6 Lot 3004	Purity: 98.8% Vehicle: 0.5 % tylose 2000 mg/kg bw Single dose under a semiocclusive dressing, 24 h	No mortalities No clinical signs of toxicity No effects on the skin No pathological findings **LD_{50} > 2000 mg/kg bw**	Anonymous, 1998 1998/11001

Acute inhalation toxicity Equivalent to OECD TG 403 (1981) GLP compliant Wistar rats 5 animals/sex/dose N6 Lot 3004	Purity: 98.8% Dust aerosol MMAD (low and mid dose): 2.5 µm MMAD (high dose): 5.1 µm 0.51, 1.28, 5.9 mg/l Single head-nose inhalation 4-hour exposure	All animals exposed to 5.9 mg/L died during exposure. 1 male and 2 females died during or immediately after exposure to of 1.28 mg/l Low and mid dose: attempts to escape, irregular accelerated and intermittent respiration, as well as squatting posture and piloerection. No clinical signs could be detected from post exposure day 5 onward. No clinical signs were detected from day 8 onward. Necropsy of the decedent mid (1.28 mg/l) concentration animals showed agonal congestive hyperaemia. No macroscopic pathologic findings **Males: LC_{50} = 1.9 mg/l** **Females: LC_{50} = 1.3 mg/l**	Anonymous, 1997, 1998

Comparison with the criteria

According to Regulation EC No 1272/2008 a substance does not meet the classification criteria for acute oral and dermal toxicity when LD_{50} values by respective routes of exposure are higher than 2000 mg/kg bw. Dimoxystrobin at 5000 mg/kg bw did not cause mortality after dosage by oral route (table above) and therefore the classification for acute oral toxicity is not warranted. Dimoxystrobin at 2000 mg/kg bw did not cause mortality after dosage by dermal route (table above) and therefore the classification for acute dermal toxicity is not warranted. In conclusion, RAC supports the DS's proposal for **no classification of dimoxystrobin for acute and dermal toxicity.**

The CLP criteria for acute toxicity via inhalation warrant a classification in Category 4, if LC_{50} values between 1.0 and 5.0 mg/L are determined. Thus**, RAC agrees with the DS's proposal for classification of dimoxystrobin as Acute Tox. 4; H332 (Harmful if inhaled) with an ATE = 1.3 mg/L (dusts or mists).**

10.4 Skin corrosion/irritation

Table 9: Summary of table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, purity, vehicle**	**Dose levels duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
Skin irritation, OECD 404 (1992), GLP compliant	Rabbit, New Zealand White, 3 male and 3 females	Dimoxystrobin (N6 Lot 3004) Purity: 98.8% Vehicle: water	0.5 g moistened with water, single application 4 hours, semiocclusive dressing, rinsed after removal of patch	Not irritating Mean scores over 24, 48 and 72 hours: 0.0, 0.0, 0.0, 0.7, 1.0, 2.0 for erythema 0.0 each for oedema Reversibility: yes, within 8 d	Anonymous, 1998 Acute dermal irritation/corrosion in rabbits

10.4.1 Short summary and overall relevance of the provided information on skin irritation/corrosion

The average score (24 to 72 h) for dermal irritation was calculated to be 0.6 for erythema (mean individual scores at 24, 48 and 72 h: 0.0, 0.0, 0.0, 0.7, 1.0 and 2.0) and 0.0 for oedema. No evidence of skin irritation was seen at 8 days.

10.4.2 Comparison with CLP criteria

The criteria to classify a substance into the category 2 for skin irritation are:

1. Mean value of ≥ 2.3 - ≤ 4.0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or

2. Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or

3. In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

Based on the mean individual scores of 0.0, 0.0, 0.0, 0.7, 1.0, 2.0 for erythema and 0.0 each for oedema, dimoxystrobin does not meet the classification criteria for skin corrosion/irritation.

10.4.3 Conclusion on classification and labelling for skin irritation/corrosion

Not classified – Conclusive but not sufficient for classification.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

DS proposed no classification of dimoxystrobin based on an OECD TG 404 and GLP compliant study showing no oedema and erythema with the mean scores over 24, 48 and 72 hours being 0.0, 0.0, 0.0, 0.7, 1.0 and 2.0 in individual animals.

Assessment and comparison with the classification criteria

The table below summarises the available study for skin corrosion/irritation by dimoxystrobin.

Table: *Summary of the available animal study on skin corrosion/irritation by dimoxystrobin.*

Study	Dose level	Results	Reference
Skin irritation OECD TG 404 (1992), GLP compliant New Zealand White rabbits 3 animals/sex N6 Lot 3004	Purity: 98.8% Vehicle: water 0.5 g moistened with water Single semiocclusive application 4 hours rinsed after removal of patch	Mean scores over 24, 48 and 72 hours: 0.0, 0.0, 0.0, 0.7, 1.0, 2.0 for erythema (mean average score: 0.6) 0.0 each for oedema Reversibility: yes (within 8 days) **Conclusion: Not irritating**	Anonymous, 1998

Comparison with the criteria

The mean individual scores (24, 48 and 72 h) for erythema and oedema (table above) were below the thresholds for warranting a classification, i.e. ≥ 2.3 and ≤ 4.0 for erythema/eschar or for oedema in at least 2 of 3 (or 4 of 6) tested animals. Thus, the classification is not supported. In conclusion, RAC agrees with the DS's proposal for **no classification of dimoxystrobin for skin irritation or corrosion.**

10.5 Serious eye damage/eye irritation

Table 10: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, purity, vehicle**	**Dose levels duration of exposure**	**Results** **- Observations and time point of onset** **- Mean scores/animal** **- Reversibility**	**Reference**
Eye irritation, OECD 405 (1987), GLP compliant	Rabbit, New Zealand White, 3 male and 3 females	Dimoxystrobin (N6 Lot 3004) Purity: 98.8% Vehicle: water	46 mg, single application the conjunctival sac of the right eye, washed out after 24 h	Not irritating Mean scores over 24, 48 and 72 hours: 0.0 Each for corneal opacity 0.0, 0.0, 0.0, 0.3, 0.0, 0.0 for iris lesions, 0.0, 0.3, 0.7, 1.7, 0.7, 0.3 for redness of the conjunctiva, 0.0, 0.0, 0.0, 0.7, 0.0, 0.0 for chemosis Reversibility: yes, within 72 h	Anonymous, 1998 Acute eye irritation in rabbits

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

No signs of cornea opacity were seen at any of the observation time points. Slight conjunctival responses were seen at 1h, including grade 1-2 conjunctival discharge. The average score (24 to 72 h) for eye irritation was calculated to be 0.1 for iritis (mean individual scores at 24, 48 and 72 h: 0.0, 0.0, 0.0, 0.3, 0.0, 0.0), 0.6 for conjunctival redness (mean individual scores at 24, 48 and 72 h: 0.0, 0.3, 0.7, 1.7, 0.7, 0.3) and 0.1 for chemosis (mean individual scores at 24, 48 and 72 h: 0.0, 0.0, 0.0, 0.7, 0.0, 0.0). One animal at 24 h only showed slight conjunctival discharge and a contracted pupil. The effects were fully reversible within 72h.

10.5.2 Comparison with the CLP criteria

A substance shall be categorized for reversible eye effects (category 2):
If, when applied to the eye of an animal, a substance produces:

- At least in 2 of 3 tested animals, a positive respone of:
  - Corneal opacity ≥ 1 and/or
  - Iritis ≥ 1, and/or
  - Conjunctival redness ≥ 2 and/or
  - Conjunctival oedema (chemosis) ≥ 2
- Calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days.

Based on the reversible eye effects with mean individual scores of 0.0 each for corneal opacity, 0.0, 0.0, 0.0, 0.3, 0.0, 0.0 for iritis, 0.0, 0.3, 0.7, 1.7, 0.7, 0.3 for conjunctival redness, and 0.0, 0.0, 0.0, 0.7, 0.0, 0.0 for chemosis, dimoxystrobin is not to be classified for serious eye damage/irritation.

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Not classified – conclusive but not sufficient for classification.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

DS proposed no classification of dimoxystrobin based on an OECD TG 405 and GLP-compliant study showing the average individual scores of corneal opacity, iritis, conjunctival redness and chemosis at 24, 48 and 72 hours after installation of the test substance below the thresholds for warranting a classification.

Comments received during consultation

No comments were received during the consultation.

Assessment and comparison with the classification criteria

The table below summarises the available study for serious eye damage/irritation by dimoxystrobin.

***Table**: Summary of the available animal study on serious eye damage/irritation by dimosxystrobin.*

Study	Dose level	Results	Reference
Eye irritation OECD TG 405 (1987) GLP compliant New Zealand White rabbits 3 animals/sex N6 Lot 3004	Purity: 98.8% Vehicle: water 46 mg Single application the conjunctival sac of the right eye, washed out after 24 h	Mean scores over 24, 48 and 72 hours (corneal opacity): 0.0 (average score 0) Mean scores over 24, 48 and 72 hours (iris lesions): 0.0, 0.0, 0.0, 0.3, 0.0, 0.0 (average score 0.1) Mean scores over 24, 48 and 72 hours (redness of conjunctiva): 0.0, 0.3, 0.7, 1.7, 0.7, 0.3 (average score 0.6) Mean scores over 24, 48 and 72 hours (chemosis): 0.0, 0.0, 0.0, 0.7, 0.0, 0.0 (average score 0.1) **Conclusion: Not irritating**	Anonymous, 1998

Comparison with the criteria

The mean individual scores (at 24, 48 and 72 h) for corneal opacity, iritis, conjunctival redness and chemosis are below the thresholds for warranting a classification, i.e. ≥ 1 for corneal opacity and/or chemosis and/or ≥ 2 for conjunctival redness and/or oedema at least in 2 of 3 tested animals. In conclusion, RAC agrees with the DS's proposal for **no classification of dimoxystrobin for serious eye damage/irritation.**

10.6 Respiratory sensitisation

10.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

No data.

10.6.2 Comparison with the CLP criteria

The potential of dimoxystrobin to cause respiratory sensitisation was not investigated directly, as there are no recognised and validated animal tests for respiratory sensitisation. There are no data available considering the human evidence. No evidence of a respiratory tract irritation can be seen from the necropsy results of the animals treated in the acute inhalation toxicity study.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

Not classified – No data available.

10.7 Skin sensitisation

Table 11: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, purity, vehicle**	**Dose levels duration of exposure**	**Results**	**Reference**
Skin sensitisation, Maximisation Test, OECD 406 (1992), GLP compliant Deviations: No SLS pre-treatment conducted before dermal induction	Guinea pig, Pirbright White, Dunkin Hartley, Female, Each 10 controls, 20 treated groups	Dimoxystrobin (N6 Lot 3004) Purity: 98.8% Vehicle: 1% Tylose	Intradermal induction: 5% in Freund's adjuvant /0.9% aq. NaCl (1:1) Percutaneous induction: 50% in 1% tylose Challenge: 50% in 1% tylose	0/20 animals showed a response Not skin sensitising	Anonymous, 1998 Maximisation Test based on the method of Magnusson and Kligman in guinea pigs
Skin sensitisation, local lymph node assay OECD 429 (2010) GLP compliant	Mouse, CBA/CaOlaHsd Female, 5/group	Dimoxystrobin formulation BAS 540 01 F Purity: Dimoxystrobin: 199.8 g/L Vehicle: Pluronic (1%v/v)	Vehicle, 25%, 50%, 100% dilution in vehicle Topical application to the dorsal part of ear: 25 µl/day for 3 days At day 6: Injection of 19.5 µCi 3H methyl thymidine Sacrifice after 5 h	Results for vehicle/25%/50%/ 100% dose groups Stimulation indices (SI) of cell count: 1.00/1.44/1.12/1.34* [cut-off value is 1.55] SI of 3HTdR incorporation: 1.00/1.05/1.01/1.93* [cut-off value is 3.0] SI of Lymph node weight: 1.00/1.11/1.09/1.34* SI of Ear weight: 1.00/1.01/1.04/1.15 [cut-off value is 1.1, but high dose result was triggered by one individual animal] Not skin sensitizing	Anonymous, 2015a skin sensitisation: Local Lymph Node Assay

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

In the Maximization study using guinea pigs, well defined skin responses were seen after intradermal induction, where the test substance and or Freunds had been injected. After dermal induction, incrustation (caused by intradermal injection), well defined erythema and slight oedema were seen in test and control animals. After challenge no skin responses were seen in test or control animals. Dimoxystrobin is not a skin sensitizer according to results from the maximization study.

For the determination of potential sensitizing properties of the test item dimoxystrobin-containing formulation (BAS 540 01 F) the Murine Local Lymph Node Assay (LLNA) was conducted. Groups of 5 female mice were treated with three different concentrations of the test substance (25, 50, and 100% (w/w) in Pluronic® Water) or with the vehicle alone for three consecutive days.

A skin sensitizing effect for dimoxystrobin-containing formulation (BAS 540 01 F) is not considered since Stimulation Indices (S.I.) of 1.05, 1.01 and 1.93 were determined with dimoxystrobin-containing formulation at concentrations of 25, 50, and 100% in Pluronic® Water, respectively, which stay below the cut-off value of ≥ 3 for ^{3}H-thymidine incorporation. Likewise, no biologically relevant increase in lymph node cell count (S.I. of 1.44, 1.12, and 1.34) was observed in any dose group in comparison to the vehicle control group. The cut-off value for a positive response regarding the lymph node cell count index (1.55) was not exceeded. A statistically significant but biologically not relevant increase in lymph node weights was observed in the high dose group in comparison to the vehicle control group. A biologically relevant or statistically significant increase in ear weights was not observed. The cut-off value (1.1) of the ear weight index for a positive response regarding ear skin irritation (as reported for BALB/c mice) was exceeded in the high dose group (index of 1.15). This increased index was caused by the single heightened ear weight of animal No. 20, probably induced by the scratch wound, as the other four animals in this dose group had lower ear weights. No EC3 value was calculable (as no tested concentration induced a stimulation index for ^{3}H-thymidine incorporation greater than the threshold of 3. No signs of systemic toxicity and no mortalities were observed. No erythema was observed in any of the animals in the main experiment. Substance residues were reported in the animals treated with the test item. Hair loss and scratch wound was observed in one animal of the high dose group at day 6. Positive control studies performed twice a year with the sensitizer alpha-hexylcinnamaldehyde proved the sensitivity of the method used.

In conclusion, based on the results of this study dimoxystrobin-containing formulation (BAS 540 01 F) does not display skin sensitizing properties under the conditions of the test.

10.7.2 Comparison with the CLP criteria

As none of the animals in the GPMT study were showing a positive response after challenge with dimoxystrobin and no EC_3 value was calculable (as no tested concentration induced a stimulation index for 3H-thymidine incorporation greater than the threshold of 3) for the LLNA conducted with dimoxystrobin-containing formulation (which contains 200 g/L dimoxystrobin), dimoxystrobin shall not be classified for skin sensitisation.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Not classified – conclusive, but not sufficient for classification.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

DS proposed no classification of dimoxystrobin based on a skin sensitisation guinea pig maximisation test (GPMT) showing no positive response in any animal and on a skin sensitisation local lymph node assay (LLNA) showing a stimulation index lower than 3.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The table below summarises the available studies for skin sensitisation by dimoxystrobin.

***Table**: Summary of the animal studies on skin sensitisation by dimoxystrobin.*

Study	Dose level	Results	Reference
Skin sensitisation OECD TG 406 (1992) GLP compliant Pirbright White female Guinea pig 10 controls 20 treated groups N6 Lot 3004	Purity: 98.8% Vehicle: 1% tylose Intradermal induction: 5% in Freund's adjuvant /0.9% aq. NaCl (1:1) Percutaneous induction: 50% in 1% tylose Challenge: 50% in 1% tylose	0/20 animals showed a positive response Separate tests using alpha-hexylcinnamaldehyde as a positive control are conducted twice a year to determine the ability of the test to detect sensitising compounds. **No skin sensitisation**	Anonymous, 1998
Skin sensitisation, local lymph node assay OECD TG 429 (2010) GLP compliant CBA/CaOlaHsd female mice 5/group BAS 540 01 F	199.8 g dimoxystrobin/l Vehicle: Pluronic (1%v/v) Vehicle, 25%, 50%, 100% dilution in vehicle Topical application to the dorsal part of ear: 25 µl/day for 3 days At day 6: Injection of 19.5 µCi ^{3}H methyl thymidine Sacrifice after 5 h	<u>Results for vehicle/25%/50%/ 100% dose groups</u> Stimulation indices (SI) of cell count: 1.00/1.44/1.12/1.34* SI of ^{3}HTdR incorporation: 1.00/1.05/1.01/1.93* SI of lymph node weight: 1.00/1.11/1.09/1.34* SI of ear weight: 1.00/1.01/1.04/1.15 Positive control studies performed twice a year with	Anonymous, 2015a

		the sensitizer alpha-hexylcinnamaldehyde proved the sensitivity of the method used **No skin sensitisation**

Comparison with the criteria

None of the animals in the GPMT study showed a positive response after a challenge with dimoxystrobin and no tested concentration induced a stimulation index greater than the threshold of 3 in the LLNA conducted with a dimoxystrobin formulation containing 200 g/l dimoxystrobin. Therefore, classification is not warranted and RAC agrees with the DS's proposal for **no classification of dimoxystrobin as a skin sensitiser.**

10.8 Germ cell mutagenicity

Not assessed in this CLH dossier.

10.8.1 Conclusion on classification and labelling for germ cell mutagenicity

The substance is not assessed for germ cell mutagenicity in this CLH dossier.

10.9 Carcinogenicity

Not assessed in this CLH dossier.

10.9.1 Conclusion on classification and labelling for carcinogenicity

The substance has a harmonized classification and labelling as Carc. 2; H351. Carcinogenicity is not assessed in this CLH dossier.

10.10 Reproductive toxicity

The reproduction toxicity of dimoxystrobin was investigated in the following studies:

- 2-generation reproduction toxicity study in rats: groups of 25 males and 25 females, dietary concentrations 0 ppm, 50 ppm (5 - 8 mg/kg bw), 150 ppm (15 - 22 mg/kg bw), 500 ppm (47 - 75 mg/kg bw**)**, or 1200 ppm (108 - 168 mg/kg bw) (Anonymous 2001a, 2000/1016869)
- Modified one-generation toxicity study in rats: groups of 10 males and 10 females, dietary concentrations of 0 ppm, 150 ppm (18 mg/kg bw/day), 500 ppm (57 mg/kg bw/day), or 1200 ppm (130 mg/kg bw/day), additional haematological examinations to investigate anemia (Anonymous 2001 b, 2000/1016870)
- Enhanced one-generation reproduction toxicity in rats: groups of 25 males and 25 females, dietary concentrations of 0 ppm, 10 ppm (0.9 mg/kg bw/day), 20 ppm (1.7 mg/kg bw/day), and 50 ppm (4.3 mg/kg bw/day) (Anonymous 2011a, 2011/1211676). In this study a clear no observed adverse effect level (NOAEL) for haematological effects for both adults and pups was determined
- Prenatal toxicity studies in rats (Anonymous 1999a, 1999/11680) and rabbits (Anonymous 2001a, 2000/1016867, 2001/1016351)

The following additional calculations, mechanistic studies, and historical control data have been summarized below and were considered:

- Benchmark dose calculations of $BMDL_{05}$ levels for body weight changes in F1 adult females and F2 pups during lactation in the 2-generation reproduction study (Dammann M (2015):)
- Mechanistic studies to investigate the effects of dimoxystrobin on haematological parameters and iron metabolism in rats (Anonymous 2002a, 2002/1005354, 2002/1014245, Anonymous 2005a 2005/1004845)
- Mechanistic study in 3-week old rats (Anonymous 2010a, 2010/1026748), to determine a NOAEL for effects on serum iron levels
- Historical control data from 2-generation toxicity studies conducted in the BASF laboratories between April 2000 and September 2002 in Wistar rats (Charles River) (Anonymous 2017a, 2017/1201528)
- Historical control data from rabbit (Himalayan strain) developmental toxicity studies conducted in the BASF laboratories between April 1999 and November 2003 (Anonymous 2013a, 2013/1421980)

The benchmark dose calculation for the 2-generation study (Dammann M (2015)), the enhanced one-generation study (Anonymous 2011a, 2011/1211676) and the mechanistic study in 3-week old rats (Anonymous 2010a, 2010/1026748) are new data that were submitted in Europe for the renewal of approval of dimoxystrobin in order to demonstrate that current harmonised classification of dimoxystrobin as to Repro. Cat. 2, H361d is not warranted and provide sufficient information for the re-classification process.

10.10.1 Adverse effects on sexual function and fertility

Table 12: Summary table of animal studies on adverse effects on sexual function and fertility (complete study details are summarized)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Two-generation reproductive	Test substance: Dimoxystrobin,	**1200 ppm (M: 109 mg/kg bw; F, premating: 119; gestation: 103; lactation: 168 mg/kg bw/day) F0**	Anonymous, 2001

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
toxicity study OECD 416 (Draft 1996); 87/302/EEC Part B, L 133; EPA OPPTS 870.3800; JMAFF Deviations: sexual maturation data did not include the body weight at the day of criterion; anogenital distance of F2 pups was not determined; thyroid weight of the parental animals was not determined GLP: yes Species/strain: Wistar rats Sex: male/female No. animals/sex/ dose: 25	purity: 98.4% (equivalent to substance in Chapter 1.1) Dose/concentration: 0, 50, 150, 500, 1200 ppm Route of administration: oral in feed Duration of treatment: <u>F0:</u> ♂: 74 days prior mating, up to 2 weeks mating period ♀: 74 days prior mating, up to 2 weeks mating period, continuously exposed during gestation up to weaning (lactation day LD 21) <u>F1:</u> ♂: from weanling for at least 76 days, up to 2 weeks mating period ♀: from weanling for at least 76 days, up to 2 weeks mating period, continuously exposed during gestation up to weaning (LD 21) Frequency of treat-ment: daily, 7 days/week Vehicle: none Control: yes, plain diet	**adults** <u>parental toxicity:</u> reduced food consumption, impaired body weight and body weight gain both in males and females; no treatment-related clinical observations were detected; no treatment-related mortality was observed <u>sexual function and fertility</u>: male and female reproductive performance was not affected by treatment; no effects on oestrous cycle were observed **1200 ppm (M: 156 mg/kg bw; F premating: 159, gestation: 108; lactation 168 mg/kg bw) F1 adults** <u>parental toxicity:</u> reduced food consumption, impaired body weight and body weight gain both in males and females; no treatment-related clinical observations were detected; no treatment-related mortality was observed <u>sexual function and fertility</u>: decreased number of implantation sites (within historical controls); male and female reproductive performance was not affected by treatment; no effects on oestrous cycle were observed **500 ppm (M: 47 mg/kg bw; F premating: 50, gestation: 44; lactation 75 mg/kg bw; F0 adults** <u>parental toxicity:</u> reduced food consumption, impaired body weight and body weight gain in females <u>sexual function and fertility:</u> male and female reproductive performance was not affected by treatment; no effects on oestrous cycle were observed **500 ppm (M: 62 mg/kg bw; F premating: 64, gestation: 46; lactation 75 mg/kg bw) F1 adults** <u>parental toxicity:</u> reduced food consumption, impaired body weight and body weight gain both in males and females; <u>sexual function and fertility:</u> male and female reproductive performance was not affected by treatment; no effects on oestrous cycle were observed **150 ppm (M: 14 mg/kg bw; F premating: 16, gestation: 14; lactation 22 mg/kg bw;): F0 adults** <u>parental toxicity:</u> no treatment-related effect <u>sexual function and fertility:</u> no treatment-related effect **150 ppm (M: 18mg/kg bw; F premating: 19, gestation: 14; lactation 22 mg/kg bw): F1 adults**	2000/1016869

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		parental toxicity: no treatment-related effect sexual function and fertility: no treatment-related effect **50 ppm (F0 M: 5 mg/kg bw; F premating: 5, gestation: 5; lactation 8 mg/kg bw; F1 M: 6 mg/kg bw; F premating: 6, gestation: 5; lactation 7 mg/kg bw): F0 and F1** parental toxicity: no treatment-related effect **NOAEL (Reproduction performance and fertility):** 1200 ppm (136 mg/kg bw/day) **NOAEL (Systemic toxicity (parents):** 150 ppm (17 mg/kg bw/day)	
Modified one-generation reproductive toxicity study OECD 415 (1983); 87/302/EEC Part B, L 133 Deviations: only 10 animals/sex/generation were used; exposure before mating was shorter than 70 days; haematology was included for blood samples of parental animals taken before the mating period and before sacrifice as well as of pups on PND 21 GLP: yes Species/strain: Wistar rats Sex: male/female No. animals/sex/dose: 10	Test substance: Dimoxystrobin, purity: 98.4% (equivalent to substance in Chapter 1.1) Dose/concentration: 0, 150, 500, 1200 ppm Route of administration: oral in feed Duration of treatment: ♂: 47 days prior mating, up to 2 weeks mating period ♀: 47 days prior mating, up to 2 weeks mating period, continuously exposed during gestation up to weaning (LD 21) Control: yes, plain diet	**1200 ppm (130 mg/kg bw/day):** parental toxicity: reduced food consumption, impaired body weight gain, microcytic hypochromic anaemia sexual function and fertility: no treatment-related effect **500 ppm (57 mg/kg bw/day):** parental toxicity: reduced food consumption, impaired body weight gain, microcytic hypochromic anaemia sexual function and fertility: no treatment-related effect **150 ppm (18 mg/kg bw/day):** parental toxicity: microcytic hypochromic anaemia (slight) sexual function and fertility: no treatment-related effect NOAEL (Systemic toxicity, parents): not obtained in this study, is considered to be 50 ppm as observed in the previous 2-generation study above (5 mg/kg bw/day)	Anonymous, 2001b 2000/1016870
Enhanced one-generation reproductive toxicity study	Test substance: Dimoxystrobin, purity: 98.5% (equivalent to	**50 ppm (4.3 mg/kg bw/day):** parental toxicity: no treatment-related effect sexual function and fertility: no treatment-related	Anonymous, 2011 2011/1211676

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
OECD 416 (2001); 2008/440/EC, Part B, L 142; EPA 870.3800 Deviations: study design was limited to 1 generation; estrus cycle and sperm parameters were not determined; organ weight determination, gross necropsy and histopathology were not included; haematology and determination of iron and transferrin was included for blood samples of parental animals taken before sacrifice as well as of pups on PND 7, 14, 21 GLP: yes Species/strain: Wistar rats Sex: male/female No. animals/sex/ dose: 25	substance in Chapter 1.1) Dose/concentration: 0, 10, 20, 50, ppm Route of administration: oral in feed Duration of treatment: ♂: 73 days prior mating, up to 2 weeks mating period ♀: 73 days prior mating, up to 2 weeks mating period, continuously exposed during gestation up to weaning (LD 21), however, during lactation exposure levels were reduced by 50% due to increased food consumption during that phase Control: yes, plain diet	effect **20 ppm (1.7 mg/kg bw/day):** parental toxicity: no treatment-related effect sexual function and fertility: no treatment-related effect **10 ppm (0.9 mg/kg bw/day):** parental toxicity: no treatment-related effect sexual function and fertility: no treatment-related effect **NOAEL (Reproduction performance and fertility):** **50 ppm (4.3 mg/kg bw/day)** **NOAEL (Systemic toxicity (parents):** **50 ppm (4.3 mg/kg bw/day)**	

Table 13: Summary table of human data on adverse effects on sexual function and fertility

No data available.

Table 14: Summary table of other studies relevant for toxicity on sexual function and fertility

No data available.

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

In this chapter only the effects (as summarized in the above table) possibly related to sexual function and fertility are discussed. All effects – relevant for the discussion of developmental toxicity – are discussed in Chapter 10.10.5.

In the 2-generation study parental toxicity was indicated by impairment of food consumption, body weight and body weight gain ≥ 500 ppm.

Food consumption was reduced to about 4% in high dose (1200 ppm) F0 parental male animals during the entire premating period (statistically significant at weeks 7-8 and 9-10). During pre-mating food consumption of the high dose (1200 ppm) F0 parental females was only slightly below the corresponding controls (about 4%). During gestation and lactation however food consumption in this group was about 8-13% lower compared to the control group, which also had an effect on the body weight of dams and was thus considered substance-related. Food consumption of all other treated F0 males and females was not affected by treatment. The impairments of food consumption of the high dose F0 parental animals were considered to be treatment-related, due to concurrent impairments in body weight/body weight gain in these rats.

Food consumption was statistically significantly reduced in the 500 (during most weeks) and 1200 ppm parental F1 male animals during pre-mating. Food consumption over the entire pre-mating period was impaired by about 8 or 16% compared to the concurrent control. This is in-line with the impaired body weights at 500 and 1200 ppm. Food consumption in 500 and 1200 ppm F1 parental females was reduced during the whole premating period, but less pronounced compared to males. Food consumption in these dose groups was further impaired during gestation and lactation attaining statistical significance during several intervals. Compared to the corresponding controls food consumption at 500 ppm was about 4, 6, and 10%, that of the 1200 ppm dose group about 12, 18, and 26% lower over the entire pre-mating, gestation and lactation phase. Food consumption of all treated 50 ppm and 150 ppm F1 males and females was not affected by treatment.

As a general comment with regard to systemic toxicity, it must not be forgotten, that the reproduction toxicity studies with dimoxystrobin were conducted without dose adjustment during lactation, which means, that the actual substance intake was much higher in females compared to males (at 1200 ppm males consumed 109 mg/kg bw and females consumed 168 mg/kg bw) and also significantly higher in the F1 adult compared to the F0 adult female generation (1200 ppm: substance intake during premating: F0: 119 mg/kg bw; F1: 159 mg/kg bw).

In the modified one-generation study parental toxicity indicated by impairment of food consumption, body weight and body weight gain ≥ 500 ppm and microcytic hypochromic anaemia ≥ 150 ppm.

At termination of the study the mean body weight of the 500 ppm males was about 11 % that of the 1200 ppm males about 17% below the corresponding control value. This is in-line with the concurrent reductions in food consumption in these groups. Mean body weight gain of the mid and high dose F0 males were also clearly impaired; if calculated for study weeks 0 - 13, the mean weight gain in the mid dose group was about 14 %, that of the high dose group about 24% below the respective control value. The mean body weights and mean body weight gains of the 150 ppm F0 males were substantially similar to control values.

Mean body weights of the substance-treated F0 females were substantially similar to controls during the premating period, but the weight gain of the high dose females (1200 ppm) was about 11% below the corresponding control value if calculated for weeks 0 – 6.

During gestation the mean body weights and body weight gains of the high dose F0 parental females were also impaired. The mean body weight of the 1200 ppm dams on day 20 p.c. was about 9%, the body weight gain from days 0 - 20 p.c. was about 18% below the corresponding control value.

During the lactation period the mean body weights of the 1200 ppm females were also below the corresponding control values (up to about 12% on day 14 p.p.), the differences, however, did not always reach statistical significance. The mean body weight gains of the substance-treated females did not show a consistent trend throughout the lactation period; particularly at the high dose, the weight gains were sometimes distinctly above, sometime distinctly below the corresponding control values. Mean body weights and mean body weight gains of the 150 and 500 ppm females were not affected by the test substance administration during premating, gestation or lactation, particularly if the normal range of biological variation is taken into consideration.

In the F0 parental male animals of the mid and high dose group (500 or 1200 ppm) the feed consumption was statistically significantly reduced during the premating period (study weeks 0 - 6); if calculated for the entire premating period the food intake was about 11% (500 ppm) or 19% (1200 ppm), respectively, below the corresponding control value. This is considered to reflect a substance-related effect as body weight data of the mid and high dose males were concomitantly affected. The food intake of the low dose males, however, was similar to control values.

The feed consumption of the high dose F0 parental females was statistically significantly reduced during the premating period (study weeks 0 - 6); if calculated for the entire premating period, the feed intake was about 12% below the corresponding control value.
During gestation and lactation of the F1 litter, food consumption of the 1200 ppm F0 dams was also statistically significantly impaired and on average about 14 - 20% lower than in the control group. As the impairments in the dams' feed intake at the high dose had also some concurrent effects on the dams' body weight data, these effects are considered as substance - related. The food consumption of the 150 and 500 ppm female F0 parental rats during premating, gestation and lactation did not show any statistically significant differences and was generally comparable to that of the controls taking the normal range of biological variation into consideration.

In the enhanced one-generation study mean body weights and body weight gain of the mid- and high-dose F_0 males (20 and 50 ppm) were comparable to the concurrent control group throughout the. However, high-dose body weight was slightly, but statistically significantly, reduced in week 6. Furthermore, F_0 males gained either less body weight during study weeks 0-1 and weeks 3-5 (20 and 50 ppm test groups) or more body weight during weeks 13-14 (20 ppm test group).
However, low-dose parental males had statistically significantly lower body weights from study week 5 onwards until the end of the study in week 15. Their body weight gain was statistically significantly decreased during weeks 0-5. During the entire study, the low-dose males gained about 8% less weight than in controls.
All statistical significant differences noted during isolated periods between the substance treated groups and the concurrent control are without biological relevance and considered not treatment-related due to the lack of dose-response relationship.
Neither mean body weights nor mean body weight gain of the F_0 parental females in all dose groups were influenced by the test substance during premating, gestation and lactation periods. The statistically significantly increased body weight gain during GD 0-7 (10 and 20 ppm test groups) and during PND 7-14 (20 ppm test group) was considered as spontaneous.
Food consumption of the F_0 male and female animals in all test groups (10, 20, and 50 ppm) was generally comparable to the concurrent control group during the entire study, covering premating, gestation and lactation periods.

In the 2-generation study treatment with dimoxystrobin had no effects on the estrous cycle, the number, morphology and motility of sperm as well as on male or female fertility. Sperm parameters of dimoxystrobin were determined in F0 and in F1 generation. In the F0 generation the mean sperm counts/g testes and /g cauda epididymides were fully within the mean historical values. The percentages for abnormal sperm were 1.7, 2.8, 3.1, 1.6 and 7.0% (historical control data: range of study means: 1.5 – 4.6% and range of individual values were 0.0 – 18.5%). Also, the sperm motility was slightly below the range of historical mean values in the high dose group with incidences of 90, 90, 92, 88 and 83% in controls, 50, 150, 500 and 1500 ppm groups (historical control data: range of study means: 86 – 93% and range of individual values were 65 – 99%). All of these parameters were also determined in the F1 generation and none of the parameters was affected. As the life-long substance intakes were higher in the F1 generation compared to the F0 generation and the exposure period was longer, this gives strong indication, that the observed changes in mean values of %abnormal sperm and %sperm motility are spurious findings and not treatment-related. Furthermore, the number of animals in the historical control data base is not very large and comprises only 5 studies, as notifiers reproduction toxicity laboratory switched from the Wistar Thomae strain used in this 2-generation toxicity study to the Charles River strain in 2000/2001 and the parameter spermatology was only implemented as a standard parameter in the years 1998/1999. Furthermore, the individual ranges for %sperm motility and %abnormal sperms are large (65 – 99% for %sperm motilities and 0 – 18.5% for %abnormal sperms).

Male and female mating and fertility indices ranged from 96% to 100% and from 80% to 100%, respectively, without any dose-response relationship.

Table 15: 2-generation reproductive toxicity study: reproduction parameters of parental animals

Parental generation	**F0**					**F1**				
Dose [ppm]	**0**	**50**	**150**	**500**	**1200**	**0**	**50**	**150**	**500**	**1200**
Male fertility										
Mating index [%]	96	100	100	100	100	96	96	96	100	96
Fertility index [%]	96	92	100	88	96	92	80	92	100	88
Female fertility										
Mating index [%]	96	100	100	100	100	96	96	96	100	96
Fertility index [%]	100	92	100	88	96	96	83	96	100	92

Dimoxystrobin treatment did not affect the reproductive performance as was evident from the absence of effects on the pre-coital interval or gestation lengths as well as gestation index (96 to 100%). In the second generation, the mean number of implantations and consequently the number of delivered pups was statistically significantly decreased compared to controls at 1200 ppm in the F1 dams (F2 litters). Historical control data for this parameter in this laboratory using this strain/breeder of rats (Hood, 2006) refers to ranges between 11.5 – 18.3 for implantation sites/dam, however the related parameter "pups delivered per dam" was likewise statistically significantly decreased in the F1 high dose females, but within historical controls. F0 females showed no such effects.

Table 16: Selected reproductive parameters from 2-generation reproductive toxicity study of dimoxystrobin (F0 Generation and F1 Litters)

PPM in Diet	**0**	**50**	**150**	**500**	**1200**
Pre coital interval (days)	3.1	2.6	2.8	2.6	2.7
Duration of gestation (days)	21.9	21.9	21.8	21.9	21.9
Gestation index (%)	100	100	100	100	100
Mean number implantations	15.0	15.4	15.8	15.4	15.1

**$p \leq 0.01$

Table 17: Selected reproductive parameters from 2-generation reproductive toxicity study of dimoxystrobin (F1 Generation and F2 Litters)

PPM in Diet	**0**	**50**	**150**	**500**	**1200**
Pre coital interval (days)	2.2	2.5	2.3	2.6	2.7
Duration of gestation (days)	21.9	21.7	21.6	21.6	21.7
Gestation index (%)	100	100	96	100	100
Mean number implantations[a)]	16.4	15.4	16.5	14.0	**12.9***

*$p \leq 0.05$

[a)]Public literature (Hood, 2006) refers to a range between 11.5 and 18.3 implantation sites/dam

The high dose effects on "implantation sites/dam" with the related parameter "pups delivered/dam", in the 2-generation study with dimoxystrobin were not consistent between the first and the second generation and can conclusively be explained by data compiled from historical control data generated for the same strain/breeder, same laboratory, similar time frame for the parameter "pups delivered/dam". Furthermore, comparable findings were not observed in the modified one-generation study applying the same dose levels, however in this mechanistic study only 10 animals were used. A short summary of the respective parameters of the modified one-generation toxicity study (Anonymous 2001b, 2000/1016870) is given below:

Table 18: Female reproduction and delivery data of the modified one generation study (Anonymous 2000/1016870)

PPM in Diet	**0**	**150**	**500**	**1200**
Pre coital interval (days)	2.8	3.2	2.7	3.7
Duration of gestation (days)	21.8	22.0	22.0	21.7
Gestation index (%)	100	100	90	100
Mean number implantations	12.0	12.3	11.0	10.3

Table 19: Female reproduction and delivery data of the enhanced one generation study (2011/1211676)

PPM in Diet	**0**	**10/5[a)]**	**20/10[a)]**	**50/25[a)]**
Pre coital interval (days)	2.9	2.4	3.0	2.5
Duration of gestation (days)	22.0	22.0	22.0	22.0
Gestation index (%)	100	100	100	100
Mean number implantations	12.9	13.5	13.2	13.2

[a)]The test concentration in the diet was reduced during lactation to account for the higher feeding rate of the dams

All the effects on parameters relevant for sexual function and fertility seen in the two generations of the 2-generation toxicity study and in the mechanistic modified 1-generation toxicity study can be regarded as incidental and not treatment-related.
The delay in vaginal opening and preputial separation in selected F1 pups, was assessed as being a consequence of the reduced body weights and not as a sign of general delay in sexual maturation. Thus, the sexual maturation of F1 offspring was not directly affected by treatment.

Gross- and histopathological examination of the reproductive organs of apparently infertile males and females did not reveal any common cause for the lack of reproductive success and thus the observed findings were considered to be unrelated to treatment. Finally, in case of ovarian follicle counts deviations from the control were regarded to be within the biological variability (increased numbers of primordial plus growing follicles and corpora lutea in the F0 generation and decreases in the number of growing follicles (with no change in combined primordial plus growing follicles) and of corporal lutea in the F1 generation; see Table 20 below).

Table 20 Ovarian follicle count in F0 and F1 maternal females

Group	Primordial	Growing	Primordial + growing	Antral	Corpora lutea
	F0 absolute numbers				
Control	2355	1021	3376	159	705
1200 ppm	2874	1284	4158	208	878
	F0 mean numbers				
Control	94	41	135	6.4	28
1200 ppm	115	51	166	8.3	35
	F1 absolute numbers				
Control	2932	1212	4135	165	650
500 ppm	3237	1345	4582	216	660
1200 ppm	3178	1063	4241	146	570
	F1 mean numbers				
Control	117	48	165	6.6	26
500 ppm	129	54	183	8.6	26
1200 ppm	127	43*	170	5.8	23*

*$p<0.05$

In the modified one-generation study male and female mating and fertility indices ranged from 90% to 100% and from 60% to 100%, respectively, without any dose-response relationship (see Table 21)

Table 21: One-generation reproductive toxicity studies: reproduction parameters of parental animals

Parental generation	**F0**			
Dose [ppm]	**0**	**150**	**500**	**1200**
Male fertility				
Mating index [%]	100	90	100	100
Fertility index [%]	60	80	100*	100
Female fertility				
Mating index [%]	100	90	100	100
Fertility index [%]	60	90	100	100

* The female mating partner of mid dose male No. 24 had only dead implants in utero at necropsy and did not deliver pups.

The occurrence of infertility in several female rats of test groups 0 and 150 ppm is assessed as being incidental and spontaneous in nature, also because no substance-induced impairments on the fertility occurred in the previous extensive two generation reproduction toxicity study.
The mean duration of gestation was very similar in all groups and the variation was negligible (between 21.7 and 22.0 days).
. Implantation was not affected by the treatment since the mean number of implantation sites was comparable between all test groups if the normal range of biological variation is taken into consideration.

In the enhanced one-generation study male mating index was 100% in all groups and fertility index was 96% in test 10 ppm group 100% in all remaining groups including the control. This reflects the normal range of biological variation inherent in the strain of rats used for this study. All respective values are within the range of the historical control data of the test facility.
The female mating index was 100% in all test groups. All sperm positive rats delivered pups or had implants in utero with the exception of low-dose (10 ppm) female #139 (mated with male #39), that did not become pregnant.
The female fertility index varied between 96% and 100%. All respective values are within the range of historical control data of the test facility and do not show any relation to dosing.
The mean duration of gestation was identical in all test groups. The gestation index was 100% in all test groups. Implantation was not affected by the treatment since the mean number of implantation sites was comparable between all test substance-related groups and the control, taking normal biological variation into account.
Thus, considering the reproduction toxicity studies, the NOAEL for sexual function and fertility was set at 1200 ppm (136 mg/kg bw/day), the NOAEL for systemic / parental toxicity was set at 150 ppm (17 mg/kg bw/day).

10.10.3 Comparison with the CLP criteria

For the purpose of classification for reproductive toxicity according to the criteria of the CLP (Regulation 1272/2008/EC), substances that are toxic to reproduction are allocated to one of two categories. Within each category, effects on sexual function and fertility, and on development, are considered separately. In addition, effects on lactation are allocated to a separate hazard category.

Category 1: Known or presumed human reproductive toxicant

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

Category 1A: Known human reproductive toxicant

The classification of a substance in Category 1A is largely based on evidence from humans.

Category 1B: Presumed human reproductive toxicant

The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

Category 2: Suspected human reproductive toxicant

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Effect on dimoxystrobin on sexual function and fertility

In the rat 2-generation reproduction toxicity study, there were no treatment-related significant effects that could be linked to impaired sexual function or fertility, such as increased time-to-mating, increased pregnancy duration, dystocia, effects on sperm parameters, adverse effects on reproductive organs, changes in the differential ovarian follicle count (DOFC), and decreases in the number of implantation sites or of litter size. All parameters were comparable with the concurrent controls or within the range of historical control data of the test facility or in case of implantation historical control data derived from published literature.

In the absence of effects on sexual function or fertility in the 2-generation toxicity study and in the enhanced one-generation toxicity study conducted according to OECD TG 416 (2001) on rats, and in the modified one-generation toxicity study conducted according to OECD TG 415 (1983) on rats no classification for sexual function and fertility is proposed.

10.10.4 Adverse effects on development

Table 22: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Prenatal developmental toxicity study OECD 414 (Draft 1996); 87/302/EEC Part B, L 133; EPA OPPTS 870.3700; JMAFF Deviations: no GLP: yes Species/strain: Wistar rats Sex: female No. animals/dose: 25 females	Test substance: Dimoxystrobin, purity: 98.8% (equivalent to substance in Chapter 1.1) Dose/concentrations: 0, 60, 120, 300 mg/kg bw/day Route of administration: oral, gavage Duration of treatment: Days 6 - 19 of gestation Frequency of treatment: daily, 7 days/week Observation period: until gestation day GD 20 Vehicle: 0.5% Tylose CB 30000 in doubly distilled water Control: yes, concurrent vehicle	**300 mg/kg bw/day:** maternal toxicity: reduced food consumption, reduction in body weight gain and in corrected body weight gain foetal toxicity: no treatment-related effect **120 mg/kg bw/day:** maternal toxicity: reduced food consumption, reduction in body weight gain and in corrected body weight gain foetal toxicity: no treatment-related effect **60 mg/kg bw/day:** maternal toxicity: no treatment-related effect foetal toxicity: no treatment-related effect **NOAEL (Maternal toxicity):** 60 mg/kg bw/day **NOAEL (Developmental toxicity):** 300 mg/kg bw/day	Anonymous, 1999 1999/11680
Prenatal developmental toxicity study OECD 414 (Draft 1996); 87/302/EEC Part B, L 133; EPA OPPTS 870.3700; JMAFF Deviations: only the head of those foetuses that showed severe abnormal findings were subject to the histopathological examinations. GLP: yes Species/strain:	Test substance: Dimoxystrobin, purity: 98.4% (equivalent to substance in Chapter 1.1) Dose/concentrations: 0, 25, 50, 100 mg/kg bw/day Route of administration: oral, gavage Duration of treatment: Days 7 - 28 of gestation Frequency of treatment: daily, 7	**100 mg/kg bw/day:** maternal toxicity: mortality (1 found dead on day 8 p.i., another was sacrificed after abortion on day 27 p.i), no defaecation, diarrhoea, reduced food consumption, impaired body weight gain foetal toxicity: 1 abortion, reduced gravid uterus weight, increased resorption rate and increased post implantation loss due to severe maternal toxicity, increased no. of fetuses with fused sternebrae (a skeletal variation) within HCD range **50 mg/kg bw/day:** maternal toxicity: no defaecation, diarrhoea, reduced food consumption, impaired body weight gain foetal toxicity: no treatment-related effect **25 mg/kg bw/day:** maternal toxicity: diarrhoea, reduced food	Anonymous, 2001 2001/1016351

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Himalayan rabbit Sex: female No. animals/dose: 25 females	days/week Observation period: until GD29 Vehicle: 0.5% Tylose CB 30000 in doubly distilled water Control: yes, concurrent vehicle	consumption, impaired body weight gain foetal toxicity: no treatment-related effect **NOAEL (Maternal toxicity):** < 25 mg/kg bw/day **NOAEL (Developmental toxicity):** 50 mg/kg bw/day	
Prenatal developmental toxicity study OECD 414 (2001); 87/302/EEC Part B, L 133; EPA OPPTS 870.3700; JMAFF Deviations: no GLP: yes Species/strain: Himalayan rabbit Sex: female No. animals/dose: 25 females	Test substance: Dimoxystrobin, purity: 98.4% (equivalent to substance in Chapter 1.1) Dose/concentrations: 0, 5, 20, 75 mg/kg bw/day Route of administration: oral, gavage Duration of treatment: GD 7 - 28 Frequency of treatment: daily, 7 days/week Observation period: until GD 29 Vehicle: 0.5% Tylose CB 30000 in doubly distilled water Control: yes, concurrent vehicle	**75 mg/kg bw/day:** maternal toxicity: mortality (2/25), no defaecation, diarrhoea, reduced food consumption, impaired body weight gain foetal toxicity: reduced gravid uterus weight, increased resorption rate and increased post implantation loss due to severe maternal toxicity, not statistically significantly increased no. of fetuses/litter with severely fused sternebrae (a skeletal malformation) within HCD and total skeletal malformations (litter incidence and affected fetuses/litter **20 mg/kg bw/day:** maternal toxicity: diarrhoea, reduced food consumption, impaired body weight gain foetal toxicity: no treatment-related effect **5 mg/kg bw/day:** maternal toxicity: no treatment-related effect foetal toxicity: no treatment-related effect **NOAEL (Maternal toxicity):** 5 mg/kg bw/day **NOAEL (Developmental toxicity):** 20 mg/kg bw/day	Anonymous, 2001 2001/1016351

Table 23: Summary table of human data on adverse effects on development

No human data available.

Table 24: Summary table of other studies relevant for developmental toxicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Two-generation reproductive toxicity study OECD 416 (Draft 1996); 87/302/EEC Part B, L 133; EPA OPPTS 870.3800; JMAFF Deviations: sexual maturation data did not include the body weight at the day of criterion; anogenital distance of F2 pups was not determined; thyroid weight of the parental animals was not determined GLP: yes Species/strain: Wistar rats Sex: male/female No. animals/sex/ dose: 25	Test substance: Dimoxystrobin, purity: 98.4% (equivalent to substance in Chapter 1.1) Dose/concentration: 0, 50, 150, 500, 1200 ppm Route of administration: oral in feed Duration of treatment: <u>F0:</u> ♂: 74 days prior mating, up to 2 weeks mating period ♀: 74 days prior mating, up to 2 weeks mating period, continuously exposed during gestation up to weaning (LD 21) <u>F1:</u> ♂: from weanling for at least 76 days, up to 2 weeks mating period ♀: from weanling for at least 76 days, up to 2 weeks mating period, continuously exposed during gestation up to weaning (LD 21) Control: yes, plain diet	**1200 ppm (M: 109 mg/kg bw; F, premating: 119; gestation: 103; lactation: 168 mg/kg bw/day) F0 adults/F1 pups:** <u>parental toxicity:</u> reduced food consumption, impaired body weight and body weight gain, increased number of females with stillborn pups <u>pup toxicity:</u> increased no. of stillborn (within historical control range), impaired body weight and body weight gain starting at PND 4, delays in development landmarks, decreased weights of thymus and spleen, hypoplasia of thymus, yellowish liver discoloration, cardiomegaly (in PND 21 pups only at necropsy; not observed in PND4 pups macroscopically) **1200 ppm (M: 156 mg/kg bw; F premating: 159, gestation: 108; lactation 168 mg/kg bw) F1 adults/F2 pups** <u>parental toxicity</u>: reduced food consumption, impaired body weight and body weight gain <u>pup toxicity</u>: Increased number of pups died (within historical control range); impaired body weight and body weight gain starting PND 4, delays in development landmarks considered secondary to body weight effects; decreased weights of thymus and spleen; yellowish liver discoloration and cardiomegaly (in PND 21 pups only at necropsy; not observed in PND4 pups macroscopically), hypoplasia of thymus (not statistically significant) **500 ppm (F0 M: 47 mg/kg bw; F premating: 50, gestation: 44; lactation 75 mg/kg bw; F1 M: 62 mg/kg bw; F premating: 64, gestation: 46; lactation 75 mg/kg bw) F0 and F1::** <u>parental toxicity:</u> reduced food consumption, impaired body weight and body weight gain <u>pup toxicity:</u> reduced no. of live born, increased no. of stillborn, reduced no. of implants, reduced no. of delivered pups (findings inconsistent between both generations), impaired body weight and body weight gain, delays in development landmarks, decreased weights of thymus and spleen, yellowish liver discoloration, cardiomegaly (in PND 21 pups only at necropsy; not observed in PND4 pups macroscopically) **150 ppm (F0 M: 14 mg/kg bw; F premating: 16, gestation: 14; lactation 22 mg/kg bw; F1 M: 18mg/kg bw; F premating: 19, gestation: 14;**	Anonymous, 2001 2000/1016869 and Dammann M (2015):

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		lactation 22 mg/kg bw): F0 and F1: parental toxicity: no treatment-related effect pup toxicity: impaired body weight and body weight gain, decreased weight of thymus, yellowish liver discoloration, cardiomegaly (in PND 21 pups only at necropsy) **50 ppm (F0 M: 5 mg/kg bw; F premating: 5, gestation: 5; lactation 8 mg/kg bw; F1 M: 6 mg/kg bw; F premating: 6, gestation: 5; lactation 7 mg/kg bw): F0 and F1:** parental toxicity: no treatment-related effect pup toxicity: no treatment-related effect **NOAEL (Systemic toxicity (parents):** 150 ppm (17 mg/kg bw/day) **NOAEL (Developmental toxicity):** 50 ppm (5 mg/kg bw/day in adults*; however, estimated actual doses in pups are 12 mg/kg bw/day due to simultaneous self-feeding*) **BMD calculations for body weight effects in F1 adult females and F2 pups:** $BMDL_{05}$ (females, PND 21): 25.5-45.3 mg/kg bw, (using measured substance intakes during lactation); $BMDL_{05}$ (pups, PND 21): 39.8 mg/kg bw (using estimated substance intakes for PND 21)	
Modified one-generation reproductive toxicity study OECD 415 (1983); 87/302/EEC Part B, L 133 Deviations: only 10 animals/sex/generation were used; exposure before mating was shorter than 70 days; haematology was included for blood samples of parental animals taken before the mating period and before sacrifice as well as of pups on PND 21	Test substance: Dimoxystrobin, purity: 98.4% (equivalent to substance in Chapter 1.1) Dose/concentration: 0, 150, 500, 1200 ppm Route of administration: oral in feed Duration of treatment: ♂: 47 days prior mating, up to 2 weeks mating period ♀: 47 days prior mating, up to 2 weeks mating period, continuously	**1200 ppm (130 mg/kg bw/day):** parental toxicity: reduced food consumption, impaired body weight gain, microcytic hypochromic anemia pup toxicity: impaired body weight gain, microcytic hypochromic anemia, increased relative heart weights, cardiomegaly (only in PND21 pups), yellowish liver discoloration, milky fluid in abdomen, pale kidneys (in PND21 pups only; not observed on PND 4 macroscopically) **500 ppm (57 mg/kg bw/day):** parental toxicity: reduced food consumption, impaired body weight gain, microcytic hypochromic anemia pup toxicity: impaired body weight gain, microcytic hypochromic anemia, increased relative heart weights, cardiomegaly (only in PND21 pups), yellowish liver discoloration, milky fluid in abdomen, pale kidneys (in PND21 pups only; not observed on PND 4 macroscopically)	Anonymous, 2001b 2000/1016870

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>GLP: yes

Species/strain: Wistar rats

Sex: male/female

No. animals/sex/ dose: 10</td>
<td>exposed during gestation up to weaning (LD 21)

Control: yes, plain diet</td>
<td>150 ppm (18 mg/kg bw/day):

<u>parental toxicity:</u> microcytic hypochromic anemia (slight)

<u>pup toxicity:</u> microcytic hypochromic anemia (slight), increased reticulocytes

<u>NOAEL (Systemic toxicity (parents):</u>

not obtained in this study, is considered to be 50 ppm as observed in the previous 2-generation study above (5 mg/kg bw/day)

<u>NOAEL (Developmental toxicity):</u>

not obtained in this study, is considered to be 50 ppm as observed in the previous 2-generation study above (5 mg/kg bw/day in adults; however, estimated actual doses in pups are 12 mg/kg bw/day due to simultaneous self-feeding)

No adverse treatment-related effects on post-implantation losses, number of pups delivered per dam, liveborn or stillborn indices:

<table>
<tr><th>PPM in Diet</th><th>0</th><th>150</th><th>500</th><th>1200</th></tr>
<tr><td>Post-implantation Loss (%)</td><td>2.8</td><td>5.3</td><td>17.5</td><td>0.8</td></tr>
<tr><td>Pups delivered per dam
(HCD 9.3 – 11.7)</td><td>11.7</td><td>11.5</td><td>10</td><td>10.2</td></tr>
<tr><td>- Liveborn</td><td>65</td><td>91</td><td>90</td><td>102</td></tr>
<tr><td>- Stillborn</td><td>5</td><td>1</td><td>0</td><td>0</td></tr>
<tr><td>Live birth index
(HCD 97 – 100)</td><td>100</td><td>85*</td><td>99</td><td>97</td></tr>
</table>
</td>
<td></td>
</tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Enhanced one-generation repro-ductive toxicity study OECD 416 (2001); 2008/440/EC, Part B, L 142; EPA 870.3800 Deviations: study design was limited to 1 generation; estrus cycle and sperm parameters were not determined; organ weight determination, gross necropsy and histopathology were not included; haematology and determination of iron and transferrin was included for blood samples of parental animals taken before sacrifice as well as of pups on PND 7, 14, 21 GLP: yes Species/strain: Wistar rats Sex: male/female No. animals/sex/ dose: 25	Test substance: Dimoxystrobin, purity: 98.5% (equivalent to substance in Chapter 1.1) Dose/concentration: 0, 10, 20, 50, ppm Route of administration: oral in feed Duration of treatment: ♂: 73 days prior mating, up to 2 weeks mating period ♀: 73 days prior mating, up to 2 weeks mating period, continuously exposed during ge-station up to weaning (LD 21), however, during lactation exposure levels were reduced by 50% due to increased food consumption during that phase Control: yes, plain diet	**50 ppm (4.3 mg/kg bw/day):** parental toxicity: no treatment-related effect pup toxicity: no treatment-related effect **20 ppm (1.7 mg/kg bw/day):** parental toxicity: no treatment-related effect pup toxicity: no treatment-related effect **10 ppm (0.9 mg/kg bw/day):** parental toxicity: no treatment-related effect pup toxicity: no treatment-related effect **NOAEL (Reproduction performance and fertility):** 50 ppm (4.3 mg/kg bw/day) **NOAEL (Systemic toxicity (parents):** 50 ppm (4.3 mg/kg bw/day) **NOAEL (Developmental toxicity):** 50 ppm (4.3 mg/kg bw/day) No adverse treatment-related effects on post-implantation losses, number of pups delivered per dam, liveborn or stillborn indices: **PPM in Diet** | **0** | **10/5[a)]** | **20/10[a)]** | **50/25[a)]** **Post-implantation Loss (%)** | 5.0 | 7.3 | 4.2 | 5.3 **Pups delivered per dam** | 12.2 | 12.5 | 12.7 | 12.6 - **Liveborn** | 305 | 294 | 318 | 313 - **Stillborn** | 1 | 6 | 0 | 2 **Live birth index** ***(HCD 97 – 100)*** | 100 | 98 | 100 | 99 [a)]The test concentration in the diet was reduced during lactation to account for the higher feeding rate of the dams	Anonymous, 2011a 2011/1211676

Table 25: Summary of mechanistic studies in rats with dimoxystrobin studying effects on hematology and iron metabolism

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Mechanistic study (no guideline avail-able) Deviations: not applicable GLP: no (appropriate data documentation,	Test substance: Dimoxystrobin, purity: 98.4% (equivalent to substance in Chapter 1.1) Dose/concentrations:	**4500 ppm:** treatment group (5 weeks: 264 mg/kg bw/day): reduced food consumption, body weight and body weight gain, evidence of hypochromic microcytic anaemia, reduced serum iron level, depletion of iron reserves recovery group (3 weeks: 232 mg/kg bw/day): The	Anonymous, 2002a 2002/1005354

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
not QA checked) Species/strain: Wistar rat Sex: male Age: adult (10 weeks) No. animals/dose: 10 males in the control group, 5 males in the treatment and recovery groups Effect on serum iron	0, 4500 ppm Route of administration: oral, diet Duration of treatment: 3 or 5 weeks Frequency of treatment: 3 weeks of treatment followed by a 2 week recovery period Control: yes, plain diet	effect on serum iron levels was fully reversible. There was some but not complete recovery in haematological effects. **NOAEL (adult rat, serum iron level):** not applicable	
Mechanistic study (no guideline available) Deviations: not applicable GLP: no (screening study) Species/strain: Wistar rat Sex: male Age: adult (10 weeks) No. animals/dose: 6 males Effect on serum iron after 2 and 6 days of dietary intake	Test substance: Dimoxystrobin, purity: 98.4% (equivalent to substance in Chapter 1.1) Dose/concentrations: 0, 10, 50, 250, 500 ppm Route of administration: oral, diet Duration of treatment: 7 days Control: yes, plain diet	**500 ppm (~40 mg/kg bw/day):** reduced serum iron level **250 ppm (~20 mg/kg bw/day):** reduced serum iron level **50 ppm (~4 mg/kg bw/day):** no treatment-related effect on serum iron level **10 ppm (~1 mg/kg bw/day):** no treatment-related effect on serum iron level **NOAEL (adult rat, serum iron level):** 50 ppm (~4 mg/kg bw/day)	Anonymous, 2002 Screening study BAS 505F: Administration I the diet and determination of serum iron after 2 and 6 days. and Amendment to the study: Screening study BAS 505F: Administration I the diet and determination of serum iron after 2 and 6 days. BASF AG, Ludwigshafen, Germany

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Mechanistic study (no guideline available) Deviations: not applicable GLP: yes Species/strain: Wistar rat Sex: male Age: young (3 weeks) and adult (10 weeks) No. animals/dose: 10 males Effect on serum iron after 7 days of dietary intake	Test substance: Dimoxystrobin, purity: 98.4% (equivalent to substance in Chapter 1.1) Dose/concentrations: young rats: 0, 250, 500 ppm old rats: 0, 500 ppm Route of administration: oral, diet Duration of treatment: 7 days Control: yes, plain diet	**500 ppm:** adult rat (33.4 mg/kg bw/day): decreased food consumption, reduced serum iron level, thickening of the duodenum in 7/10 animals young rat (65.3 mg/kg bw/day): decreased food consumption, reduced serum iron level **250 ppm:** young rat (33.8 mg/kg bw/day): decreased food consumption and body weight, reduced serum iron level **NOAEL (adult rat, serum iron level):** < 500 ppm (33.4 mg/kg bw/day) **NOAEL (young rat, serum iron level):** < 250 ppm (33.8 mg/kg bw/day)	Anonymous, 2005a 2005/1004845
Mechanistic study (no guideline available) Deviations: not applicable GLP: yes Species/strain: Wistar rat Sex: male Age: young (3 weeks) No. animals/dose: 10 males Effect on serum iron and transferrin after 7 days of dietary intake	Test substance: Dimoxystrobin, purity: 99.7% (equivalent to substance in Chapter 1.1) Dose/concentrations: 0, 6, 11, 22 ppm Route of administration: oral, diet Duration of treatment: 7 days Control: yes, plain diet	**22 ppm (3.42 mg/kg bw/day):** no treatment-related effect **11 ppm (1.71 mg/kg bw/day):** no treatment-related effect **6 ppm (0.95 mg/kg bw/day):** no treatment-related effect **NOAEL (young rat, serum iron level):** ≥ 22 ppm (3.42 mg/kg bw/day) **NOAEL (young rat, serum transferrin level):** ≥ 22 ppm (3.42 mg/kg bw/day)	Anonymous, 2010a 2010/1026748

Table 26: Summary of studies in rats using iron-deficient diet

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Public literature (no guideline study) Deviations: not applicable GLP: no Species/strain: Sprague Dawley CD rats Sex: female No. animals/dose: dams: 8 / pups: 3 males and 3 females/litter Target organ pathology in iron-deficient suckling rats	Test substance: Iron Dose/concentrations: iron-containing (307 ppm) diet, iron-deficient (5 ppm) diet Route of administration: oral in feed Duration of treatment: from GD0 until LD 18 Frequency of treatment: daily Vehicle: none Control: yes, iron containing (307 ppm) diet	**iron-deficient diet (5 ppm)** maternal toxicity: reduced body weight offspring toxicity (investigated at PND 18): reduced body weight; anemia (reduced HGB and PCV); reduced spleen size and weight; reduced thymus size; depressed hemopoiesis and lymphopoiesis; increased rel. liver weight, tan-yellow liver discoloration, fatty liver degeneration with focal hepatocellular necrosis; increased rel. kidney weight, degenerative changes of the kidney; cardiomegaly with degenerative changes **Conclusion: Iron deficiency during gestation and lactation result in morphological changes of immunocompetent tissues (spleen, thymus, liver) and impair the function of the liver, kidney and heart by hyperlipidaemia in offspring.**	Rothenbacher and Sherman, 1980
Public literature (no guideline study) Deviations: not applicable GLP: no Species/strain: Sprague Dawley CD rats Sex: male Age: 3 weeks No. animals/dose: 15 (control, treatment) - 20 (recovery) Changes in the heart of iron-deficient rats	Test substance: Iron Dose/concentrations: iron-containing (427 ppm) diet, iron-deficient (5 ppm) diet Route of administration: oral in feed Duration of treatment: 5 weeks and 5 week + 2 weeks recovery (iron-containing diet) Frequency of treatment: daily Vehicle: none Control: yes, iron containing (427 ppm) diet	**iron-deficient diet (5 ppm)** treatment group (5 weeks treatment): clinical signs (lethargy, hair loss); reduced body weight; anemia (reduced HGB); reduced succinate dehydrogenase activity of myocytes; reduced heart and serum iron level; pale liver, kidney, spleen and heart; cardiomegaly with hypertrophy of the left ventricle and papillary muscles and degenerative changes recovery group (5 weeks iron-deficient diet + 2 weeks iron-containing diet): except the heart weight and heart iron level that seemed to be still in recovery process, all other clinical parameters and body and liver weights return to normal, but the myocytes of hypertrophied left ventricles and papillary muscles still show severe degenerative changes. **Conclusion: Iron deficiency in young rats induced anemia and degenerative cardiac changes, the later still persistent after 2-weeks recovery**	Tanne et al., 1994

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Public literature (no guideline study) Deviations: not applicable GLP: no Species/strain: Sprague Dawley rats Sex: female No. animals/dose: dams: 4 - 8, fetuses: 47 - 59; pups: 18 - 80 The effects of maternal anemia on heart, placenta and body weight, and blood pressure in foetal and neonatal rats	Test substance: Iron Dose/concentrations: iron-containing (normal) diet, iron-deficient (< 6 ppm) diet Route of administration: oral in feed Duration of treatment: females: 3-4 weeks prior mating, throughout mating, gestation and lactation periods (until LD 21) Frequency of treatment: daily Vehicle: none Control: yes, iron containing (427 ppm) diet for every group	**iron-deficient diet (< 6 ppm)** maternal toxicity: no effect on systolic blood pressure foetal toxicity (GD20): reduced placental and body weight; anemia (reduced HGB) pup findings (PND 20): reduced body weight; anemia (reduced HGB); increased rel. liver, kidney and heart weights; decreased systolic blood pressure pup findings (PND 40): reduced body weight but increased body weight gain; low HGB levels raised between PND20 and PND 40 however the levels being still reduced as compared with the controls; increased rel. liver weight; high rel. kidney weight with retarded growth, increased rel. lung weight, high rel. heart weight with retarded growth; increased systolic blood pressure with dramatically increased gain from PND 20 to PND 40 **Conclusion: Maternal anemia resulted in reduced body weight, anemia, increased liver, kidney and heart weights and a low systolic blood pressure before weaning that increased thereafter**	Crowe et al., 1995

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

Prenatal toxicity studies in rats and rabbits

In the prenatal toxicity study in rats, no developmental toxicity was observed, even at the highest dose tested (300 mg/kg bw/day). Maternal toxicity was indicated by impaired food consumption and body weight development. The body weight of the litters was not affected, as well as there were no effects seen in the heart of the foetuses. Dimoxystrobin was not teratogenic in rats. The NOAEL for maternal toxicity was 60 mg/kg bw/day, whereas the developmental NOAEL was set to 300 mg/kg bw/day.

Two prenatal toxicity studies in rabbits were performed, dimoxystrobin doses were 0 25, 50 and 100 mg/kg bw in the first study and 0, 5, 20 and 75 mg/kg bw in the second study.

In the first study severe maternal toxicity was seen at 100 mg/kg bw, indicated by maternal deaths (1 female was found dead, another was sacrificed due to abortion). At ≥ 50 mg/kg bw increased incidences of no defecation was observed and the animals showed diarrhea at all dose levels. Marked to excessive, but transient drop of food consumption and body weight loss was observed at all dose levels. Calculated for the entire period high dose rabbits consumed about 15% less food, mid dose rabbits about 6% less food than concurrent controls. Mean body weight gain during treatment was significantly decreased at the high dose of 100 mg/kg (about 40%). The impaired mean body weight gains were assessed as substance-related.

At 100 mg/kg bw/day the significant maternal toxicity was considered to result in increased resorptions (mainly early resorptions) and post implantation loss (due to an increased number of early resorptions). As a consequence gravid uterus weights were lower at this dose level without attaining statistical significance.

In the second study severe maternal toxicity was seen at 75 mg/kg bw, indicated by maternal deaths (2 females died) and no defecation. At ≥ 25 mg/kg bw the incidence of diarrhoea was increased. Marked to excessive, but transient drop of food consumption and lower mean body weights / body weight gains were seen at ≥ 25 mg/kg bw. Mean food consumption fell to 75, 33 and 11% of the control for the day 7 to 8 interval at 5, 20, and 75 mg/kg, respectively. Calculated for the entire period high dose rabbits consumed about 18% less food than concurrent controls. Mean body weight gain during treatment was significantly decreased at the high dose of 75 mg/kg (about 55%) and non-statistically significantly at the mid dose of 20 mg/kg (about 19%). The impaired mean body weight gains in the mid and high dose group were assessed as substance-related.

At 75 mg/kg bw/day this maternal toxicity was considered to result in increased non-statistically significant resorptions (mainly early resorptions) and post implantation loss. As a consequence, gravid uterus weights were lower at this dose level without attaining statistical significance.

Increased incidences of post-implantation losses and mean numbers of resorptions only occurred at severely maternally toxic doses and were therefore not considered to be indicative of developmental toxicity.

The incidence of total skeletal malformations is statistically significantly increased in the second rabbit study at the 75 mg/kg bw dose, however, there were no treatment-related changes seen in the first rabbit study, which had been dosed up to 100 mg/kg bw. In the table below, the incidences for total skeletal malformations are summarized for both rabbit studies, which clearly demonstrates the absence of a dose-response. Both top doses (75 and 100 mg/kg bw) were severely maternally toxic to the rabbit does (with dramatic food consumption reduction, lower body weights, decreased to no defecation and mortalities; see below). The effects on total skeletal malformations at 75 mg/kg bw are considered incidental.

Table 27: Incidences of total skeletal malformations in both rabbit studies (Anonymous 2001a, 2000/1016867 and 2001/1016351

Parameter	**Control 1**	**Control 2**	**5**	**20**	**25**	**50**	**75**	**100**
Total skeletal malformations								
Fetal incidence [N (%)]	4 (2.6)	3 (1.8)	6 (3.6)	3 (2.0)	2 (1.2)	3 (1.9)	11 (8.1)	5 (4.8)
Litter incidence [N (%)]	3 (12)	3 (13)	6 (25)	2 (8.0)	2 (8.0)	3 (13)	9* (41)	4 (21)
Affected foetuses/litter (Mean ± SD) [%]	3.4±9.87	2.2±6.16	3.4±6.07	1.6±5.65	2.4±7.46	2.4±7.46	9.5*±14.07	6.9±16.66

*p<0.05

A summary of the maternal toxicity and relevant parameters is given in the table below.

Table 28: Key findings in the first dimoxystrobin rabbit developmental toxicity study

Mg/kg bw/day	**0**	**25**	**50**	**100**
Dam Mortality	No effect	No effect	No effect	2 (one found dead on day 8, one sacrificed after abortion on day 27)
Dam clinical signs - **Diarrhoea**	No effect	Day 8 (2 rabbits)	Day 7 – 8 (12 rabbits day 8)	Day 7 – 10 (24 rabbits day 8)
- **No defecation**		-	Day 9 (1 rabbit)	Some rabbits day 9 – 11

Dam Body weight	No effect	↓ bw gain	↓ bw gain (stat. sig.) for gestation days 7-9;	↓ bw gain (stat. sig.) for gestation days 7-9; ↓ bw gain for entire gestation period (approx. -40%)
Dam Feed consumption	No effect	↓ feed consumption (stat. sig.) for gestation days 7-8 (approx. -38%)	↓ feed consumption (stat. sig.) for gestation days 7-8 (approx. -61%); ↓ for entire gestation period (-6%)	↓ feed consumption (stat. sig.) for gestation days 7-8 (approx. -91%); ↓ for entire gestation period (-15%)
Dam Necropsy findings	No effect	No effect	No effect	No effect
Gravid uterine weight	No effect	No effect	No effect	81% (of control)
Placental weight	No effect	No effect	No effect	No effect
Dams with viable fetuses	25	25	23	19
Mean No. corpora lutea	8.0	7.9	8.5	8.0
Mean No. implant sites	7.0	6.9	7.5	7.0
Post implantation loss [%] ***(HC 5.2-20.1)***	14.2	5.0	6.6	27.5
Early resorptions [%] **-number**	11.6 0.8	3.7 0.2	5.2 0.3	25.7 2.0*
Late resorptions [%] **-number**	2.6 0.2	2.3 0.2	1.5 0.1	1.8 0.1
Mean No. Resorptions ***(HC 0.2-1.2)***	1.0	0.4	0.5	2.2*
Mean No. viable fetuses	6.0	6.5	7.0	5.5
Mean No. dead fetuses	None	None	None	None
Mean fetal body weight	38.0	38.5	37.8	38.1
Fetal sex ratio	No effect	No effect	No effect	No effect
Fetal external evaluation	No effect	No effect	No effect	No effect
Fetal visceral evaluation	No effect	No effect	No effect	No effect
Fetal skeletal evaluation	See table below			

The increased incidences of fused sternebrae, which is a frequently occurring variation in rabbits, was seen in both studies at maternally toxic doses only, and was covered by an extended historical control data (covering additionally roughly 5 years after the first studies had been conducted, see Anonymous 2013a, 2013/1421980). The finding "severely fused sternebra (bony plate)" was slightly statistically significantly increased in the second study with a litter incidence of 3. This finding was observed at the same incidence in the control of the first rabbit study and is therefore considered an incidental finding. Thus, the developmental NOAEL for rabbits was set to 50 mg/kg bw/day. There was no evidence for dimoxystrobin being teratogenic.

Table 29: Incidences of fused sternebra, severely fused sternebra and septum ventricular defects in the first and the second rabbit prenatal toxicity study

Finding	Control	Low dose	Mid Dose	High Dose
- Fused sternebra - First study				
- Fetal incidence [N (%)] *(HCD 0.0-10.7)* [1)]	2 (1.3)	9 (5.5)	10 (6.2)	11 (10)
- Litter incidence [N (%)] *(HCD 0.0-50.0%)*[1)]	2 (8.0)	**9*** (36)	**8*** (35)	**9**** (47)
- Affected fetuses/litter (Mean) [%] *(HC 0.0-13.5%)*[1)]	0.9	**5.1****	**6.5****	**15.2****[2)]
Fused sternebra - Second study				
- Fetal incidence [N (%)] *(HCD 0.0-10.7)* [1)]	5 (3.0)	8 (4.8)	3 (2.0)	16 (12)

- Litter incidence [N (%)] *(HCD 0.0-50.0%)[1)]*	4 (17)	5 (21)	2 (8.0)	8 (36)
- Affected fetuses/litter (Mean) [%] *(HCD 0.0-13.5%)[1)]*	2.7	4.6	2.5	11.2
- Membranous ventricular septum defect – Second study (not seen in first study)				
- Fetal incidence [N (%)]	1 (0.6)	0 (0.0)	1 (0.7)	3 (2.2)
- Litter incidence [N (%)] *(HCD: 0 – 17.6%)*	1 (4.2)	0 (0.0)	1 (4.0)	3 (14)
- Affected fetuses/litter (Mean) [%]	0.5	0.0	0.5	2.4
- Sternebrae severely fused (bony plate) – First study				
- Fetal incidence [N (%)]	3 (2.0)	0 (0.0)	0 (0.0)	1 (1.0)
- Litter incidence [N (%)]	3 (12.0)	0 (0.0)	1 (4.0)	1 (5.3)
- Affected fetuses/litter (Mean) [%]	2.6	0.0	0.0	1.8
- Sternebrae severely fused (bony plate) – Second study				
- Fetal incidence [N (%)]	0 (0.0)	0 (0.0)	1 (0.7)	3 (2.2)
- Litter incidence [N (%)]	0 (0.0)	0 (0.0)	1 (4.0)	3 (14)
- Affected fetuses/litter (Mean) [%]	0.0	0.0	0.5	**2.2*[3)]**

*p ≤ 0.05; ** p ≤ 0.01

[1)] extended historical control data (Anonymous 2013a, 2013/1421980) covering a time span of roughly ± 5 years around the experimental date.

[2)] when excluding dam #87 (which had 100% affected fetuses (=1 affected pup)/litter) from the evaluation the incidence decreases to 11.1%

[3)] comparable incidence as the control group of the first study

Developmental toxicity investigated in generation toxicity studies

Regarding the effects on development observed in the reproductive toxicity studies, survival of pups was considered not to be affected by the treatment (see the assessment below).

In the 2-generation study the number of F0 females with stillborn pups and thus the total number of stillborn F1 pups were slightly increased in the parental F0 generation (F1 litters) at 1200 ppm, but fully within the historical control data range. Consequently, the live birth index was slightly, but statistically significantly reduced at 1200 ppm (95%), which however was fully within the historical control data (HCD 90-99%). For details please refer to table below. These findings were not observed in the F1 females / F2 pups and are therefore unlikely to be treatment-related. In the modified one-generation study, the post implantation loss values were unaffected by treatment and did not show dose-response relationship. The gestation index was 100% for test group 0, 500 and 1200 ppm. In the test group 150 ppm the gestation index reached 90% due to the fact, that one pregnant female delivered no pups, but had only dead implants in utero. Thus, the only statistically significant decrease in live birth index was observed in the 150 ppm dose group, which is not considered to be treatment-related. In the enhanced one-generation study, there were no indications for test substance-induced intrauterine embryo or fetolethality since the post implantation loss did not show any statistically significant differences between the groups, and the mean number of F_1 pups delivered per dam remained unaffected.

Table 30: Developmental parameters from Two-Generation Reproductive Toxicity Study of Dimoxystrobin (F0 Generation and F1 Litters)

PPM in Diet	**0**	**50**	**150**	**500**	**1200**
Post-implantation loss per litter (mean %)	11.7	11.4	14.4	5.5	7.3
Number of litters	24	23	25	22	24
Females with liveborn pups	24	23	25	22	24
Females with stillborn pups (% of litters delivered) *(HC 3-13)*	2 (8.3)	6 (26)	4 (16)	3 (14)	**11*** (46)
Number of liveborn pups (Live Birth index - %) *(HCD 90-99%)*	315 (99)	296 (98)	336 (99)	314 (99)	318 **(95)****
Mean number pups delivered	13.2	13.2	13.6	14.4	14.0

Number of pups stillborn (% of number delivered) *(HC 4-35)*	2 (0.6)	7 (2.3)	5 (1.5)	3 (0.9)	**17**** (5.1)
Pups died *(HC 4-31)*	9	8	11	12	17
Pups (%) surviving Days 0-4 (Viability Index)	96	96	96	93	93
Pups (%) surviving Days 4-21 (Lactation Index)	100	98	99	98	98
Live pups (mean) PND 0	13.1	12.9	13.4	14.3	13.3
Live pups (mean) PND 21	7.7	7.4	7.5	7.5	7.6

*$p \leq 0.05$; ** $p \leq 0.01$

The large historical control ranges already indicate, that the parameters "stillborn pups" and females with stillborn pups" have a relatively high variation in this Wistar rat from the Thomae strain. In general, the Wistar rats from the Thomae strain produce bigger litters (range of mean pups delivered/dam is 11.1 – 16.4 in studies conducted between August 1992 and February 1999), than the Charles River strain (range of mean pups delivered/dam is 9.3 – 11.7 in studies conducted between May 2000 and February 2003), which possibly explains the higher number of incidental stillborn pups or perinatal pup deaths (details on the historical control data submitted in the study report are summarized in Annex I to this CLH report – 3.10.1.1 and 3.10.1.3 (Anonymous 2017a, 2017/1201528). In parts of the results from another study, where the used test substance was not equivalent to dimoxystrobin (study number 94041), conducted in the same lab with the same strain of animals in around the same time frame, are presented. The number of females with stillborn pups and number of stillborn pups in the control group in comparison with the data from the 2-generation reproductive study with dimoxystrobin indicates that there is a large biological variation of these parameters.

Table 31: Reproductive Indices and Selected Reproductive and Pup Parameters of the control groups from Two-Generation Reproductive Toxicity Study No. 94041 (F0 Generation and F1a/F1b Litter; F1 Generation and F2 Litter)*

Control group	**F1a**	**F1b**	**F2**
Females with liveborn pups	25	25	23
Females with stillborn pups (% of litters delivered)	9 (36)	13 (52)	4 (17)
Number of liveborn pups (Live Birth index - %)	310 (94)	331 (90)	320 (99)
Mean number of pups delivered	13.2	14.6	14.1
Number of pups stillborn (% of number delivered)	20 (6.1)	35 (9.6)	4 (1.2)
Pups died	11	11	21
Pups (%) surviving Days 0-4 (Viability Index)	96	94	93

*Study was conducted in Wistar rats supplied from Thomae and started in June 1995

The mean number of pups per litter alive on PND 0 was not statistically significantly lower than controls and there was no increase in stillbirths in F2 generation. The number of stillborn F2 pups was comparable between control and treated groups, and there was no effect on the live birth index. A summary table of the findings is shown below:

Table 32: Reproductive Indices and Selected Reproductive and Pup Parameters from Two-Generation Reproductive Toxicity Study of Dimoxystrobin (F1 Generation and F2 Litters)

PPM in Diet	**0**	**50**	**150**	**500**	**1200**
Post-implantation loss per litter (mean %)	9.2	11.1	13.4	10.7	8.4
Number of litters	23	20	22	25	22

Females with liveborn pups	23	20	22	25	22
Females with stillborn pups	4	7	4	4	5
Number of liveborn pups (Live Birth index - %)	338 (99)	266 (97)	322 (98)	310 (99)	253 (97)
Mean pups delivered *(HCD 11.1-16.4)*	14.9	13.8	15.0	12.6	**11.8***
Number of pups stillborn (% of pups born)	4 1.2	9 3.3	8 2.4	4 1.3	7 2.7
Pups died *(HC 4-31)*	5	10	**14***	10	**19****
Pups (%) surviving Days 0-4 (Viability Index)	97	95	96	95	92
Pups (%) surviving Days 4-21 (Lactation Index)	99	99	97	99	99
Live pups (mean) PND 0	14.7	13.3	14.6	12.4	11.5
Live pups (mean) PND 21	7.9	7.3	7.8	7.3	7.5

*$p \leq 0.05$; ** $p \leq 0.01$

The total number of F2 pups that died, was statistically significantly increased at 150 ppm and at 1200 ppm, but fully within the historical control data range. Concerning the 150 ppm dose group, no dose-relation could be detected. In the high dose group, this was predominantly caused by just one litter (eight pups of this dam (#707) died/were found dead on day 1 after delivery, which can also happen sporadically in control animals of this rat strain). This increased number of died pups in the high dose did not affect the viability index, which was with 92% still within the historical control range (HCD 83 - 99%). Therefore, the effect was considered to be spontaneous.

This can be further illustrated by the results of a further 2-generation toxicity study (Study No. 96172) performed with the same rat strain at the same lab, where the used test substance was not equivalent to dimoxystrobin. In this study, the numbers of died pups in the control group in comparison with the data from the 2-generation reproductive study with dimoxystrobin indicates that there is a large biological variation of these parameters (see table below).

Table 33: Reproductive Indices and Selected Reproductive and Pup Parameters from Two-Generation Reproductive Toxicity Study No. 96172 (F0 Generation and F1 Litters; F1 Generation and F2 litters)*

PPM in Diet	**0**	**25**	**75**	**300**
F0 Generation and F1 Litters				
Pups died	**31**	**40**	1	8
Pups (%) surviving Days 0-4 (Viability Index)	93	87	100	93
F1 Generation and F2 litters				
Pups died	7	4	11	8
Pups (%) surviving Days 0-4 (Viability Index)	97	98	95	96

* Study was conducted in Wistar rats supplied from Thomae and started in August 1998

In the 2-generation study, viability and lactation indices were in the range of 92% to 100% and 97% to 100%, respectively. The increased number of pups died during lactation, lowered the high dose lactation index in both generations, however, the parameter still being within the range of the historical control data. In the modified one-generation study, viability and lactation indices were in the range of 93% to 100% and 99% to 100%, respectively. In the enhanced one-generation study, viability and lactation indices were in the range of 97% to 99% and 49% to 55%, respectively. The lowered lactation index (in the enhanced 1-generation study) is due to the experimental design of the study and was caused by the fact that selected pups were sacrificed for blood sampling on PND 7, 14, and 21.

Table 34: Generational reproductive toxicity studies: pup survival indices

Pup generation	**F1**							**F2**				
Dose level [ppm]	**0**	**10**	**20**	**50**	**150**	**500**	**1200**	**0**	**50**	**150**	**500**	**1200**

2-generation toxicity study												
Viability index [%]	96			96	96	93	93	97	95	96	95	**92****
HCD [%]	83 - 99											
Lactation index [%]	100			98	99	98	98	99	99	97	99	99
Modified 1-generation study												
Viability index [%]	100				**93***	99	99					
HCD	83 - 99											
Lactation index [%]	100				100	99	100					
Enhanced 1-generation study												
Viability index [%]	97	98	99	99								
Lactation index [%]	54	49	55	50								

Significant effects on body weight were essentially absent at birth in the offspring animals. Therefore, an in-utero effect can be excluded. In line with maternal body weight development, mean pup body weights of F1 pups in the 500 and 1200 ppm dose test groups were statistically significantly reduced compared to controls from postnatal day (PND) 4 onward. Maternal body weights were between -4.0 -7.0% lower than controls in the 500 ppm dose group and between -5.5 - -8.9% lower in the 1200 ppm dose group. In the F1 pups the % body weight decreased compared to controls were -3.1 – -16.7% in the 500 ppm and -4.7 – -35.4% in the 1200 ppm dose group (see Table 35).

Body weight effects in the offspring became more pronounced in the later phase of lactation, especially in the last week of lactation (time points PND 14 and 21), when the pups start self-feeding (around PND12; Hood, 2011; Tyl et al., 2008, 2008/1102837). Table shows the comparison of maternal body weights (F0 generation) with pup body weights (F1 generation).

Table 35: Maternal (F0) and pup (F1) body weights during lactation

F0 maternal	**ppm**				
Day	**0**	**50**	**150**	**500**	**1200**
1	298.5	301.7	300.9	277.6* (-7.0)	278.9* (-6.6)
4	315.3	309	312.8	294.4** (-6.6)	287.2** (-8.9)
7	323.6	320.6	322.1	308.9 (-4.5)	299.2** (-7.5)
14	334.9	333.6	332	315.2* (-5.9)	305.2** (-8.9)
21	325.7	327.3	324	312.7 (-4.0)	307.7* (-5.5)
F1 litters	**ppm**				
day	**0**	**50**	**150**	**500**	**1200**
1	6.4	6.4	6.4	6.2 (-3.1)	6.1 (-4.7)
4 preculling	9.2	9.1	9.3	8.2 (-10.9)	8.1* (-12.0)
4 postculling	9.3	9.2	9.3	8.2* (-11.8)	8.1* (-12.9)
7	14.9	14.8	15.1	13.1* (-12.1)	11.9** (-20.1)
14	32	31.8	31.7	27.7** (-13.4)	23.4** (-26.9)
21	52.6	52.9	51.8	43.8** (-16.7)	34.0** (-35.4)

*$p \leq 0.05$; ** $p \leq 0.01$
In brackets (% vs. control)

It is to be expected that pups show more severe effects on body weights since they receive higher daily doses of dimoxystrobin compared to the dams at the same dietary doses. This is due to the fact that dietary exposure was continuous throughout the 2-generation study (and the supplementary modified one-generation study, see below), and dietary concentrations were not reduced during lactation. Therefore, actual maternal dose levels during the lactation phase are increased due to a physiological higher need for food. This leads in turn to a considerably higher dosing of the pups due to an assumed higher concentration in milk and additionally at the later lactational phase via self-feeding starting at around PND 12 (Hood, 2011; Tyl et al., 2008, 2008/1102837). A comparison of the actually measured maternal (F0 and F1) dimoxystrobin doses is shown in Table , including the estimated mg/kg bw/day exposures for the F1 and F2 pups during the last week of lactation only, which considers estimated direct exposure of pups via treated feed. Assuming the presence of dimoxystrobin in milk would further increase the systemic doses of offspring animals.

Table 36: Approximate mg/kg bw/day compound exposure to parental (F0 and F1) animals and estimated mg/kg bw/day exposure to F1 and F2 pups during the last week of lactation (excluding amount transferred in milk) in the 2-generation study

	Compound exposure (mg/kg bw/day)				
ppm in diet	**0**	**50**	**150**	**500**	**1200**
F0 male (premating)	0	4.7	14.1	46.4	108.8
F0 female (premating)	0	5.1	15.6	49.9	118.9
F0 Female (gestation)	0	4.5	13.6	43.6	102.5
F0 Female (lactation)[a)]	0	7.6	22.1	74.5	168.2
F1 pups[b)]	0	9.8	29.7	96.3	227.7
F1 pups (not corrected)[c)]	0	6.1	17.9	59.1	135.4
F1 Male (premating)	0	5.9	18.2	61.8	156.4
F1 Female (premating)	0	6.2	18.6	63.7	159
F1 Female (gestation)	0	4.6	13.6	46.1	107.8
F1 Female (lactation)[a)]	0	7.4	22.4	75.4	168
F2 pups[b)]	0	12.1	36.8	125.5	315.4
F2 pups (not corrected)[c)]	0	6	18	60.8	138

a) Excludes final week of lactation because of pup self-feeding

b) 2.0 fold factor for estimated pup dietary consumption on a mg/kg bw/day basis as adults through self-feeding behavior in the last week of lactation based on pre-weaning pup consumption of radiolabelled microsphere recorded by Hanley and Watanabe 1985; 1985/1002252) (weaning at PND 28), plus estimated compound consumption during late lactation supported by the dietary 2,4-D rangefinding TK study (Saghir et al., 2013; 2013/1419940). This factor was applied to the compound intake based on mean premating adult male and female feed consumption.

c) Not corrected values for pup dietary test substance intake based on approximate compound exposure to females during gestation and lactation (mean values)

The estimated values for pups' substance intake (in the last week of lactation) is considerably higher in pups compared to female adults (a dose of 227.7 mg/kg bw is estimated for F1 pups, while females consume only 168.2 mg/kg bw over the lactation period in the 1200 ppm dose group). This difference is even more pronounced in the F2 pups of the 1200 ppm dose group with an estimated test substance intake of 315.4 mg/kg bw/day compared to 168 mg/kg bw/day in the respective high dose females (Table).

These are important considerations for evaluating relative sensitivities between parental animals and offspring and demonstrate that dietary concentrations are not directly comparable between dams (or adults) and pups, and any comparisons of findings to evaluate potential pup sensitivity or relative severity of effects in the pups should be assessed to the extent possible on a mg/kg bw/day basis and not on the ppm in the diet.

A similar picture with regard to pup body weights is seen in the second generation of this 2-generation toxicity study [see Table 16]. Body weight effects are seen in the F2 pups down to the 150 ppm dose group.

Table 16: Maternal (F1) and pup body weights (F2 litters) during lactation

F1 maternal	**Body weight [g]**				
day	**0 ppm**	**50 ppm**	**150 ppm**	**500 ppm**	**1200 ppm**
1	308.7	306.0	299.9	275.8** (-10.7)	248.7** (-19.4)
4	317.9	320.3	309.6	288.4** (-9.3)	256.2** (-19.4)
7	327	328.9	319.4	299.5** (-8.4)	265.7** (-18.7)
14	347.2	345.9	342	314.9** (-9.3)	276.6** (-20.3)
21	330	330.6	330.2	313.4 (-5.0)	280.7** (-14.9)
F2 litters	**Body weight [g]**				
day	**0 ppm**	**50 ppm**	**150 ppm**	**500 ppm**	**1200 ppm**
1	6.4	6.4	6 (-6.3)	6.2 (-3.1)	6.2 (-3.1)
4 preculling	9.2	9.5	8.4 (-8.7)	9.2 (0)	8.5 (-7.6)
4 postculling	9.2	9.6	8.5 (-7.6)	9.2 (0)	8.5 (-7.6)
7	14.8	15.3	13.2* (-10.8)	14.2 (-4.1)	11.8** (-20.3)
14	31.9	32.7	29.2* (-8.5)	28.8** (-9.7)	22.3** (-30.1)
21	51.2	52.3	47.1* (-8.0)	44.3** (-13.5)	32.7** (-36.1)

*$p \leq 0.05$; ** $p \leq 0.01$
In brackets (% vs. control)

Also here, a clearly increased trend in pup body weight effects especially after PND 14 can be observed with no effects at birth. The NOAEL for pup body weight effects was 50 ppm, while the NOAEL for maternal body weight effects was 150 ppm. Although this picture seems to indicate a higher susceptibility of the pups compared to the adults, it needs to be considered, that again the actual doses between pups and adults are considerably different at the same dietary concentrations (see Table). The severity of the body weight decreases in F2 pups of the 500 ppm (=125.5 mg/kg bw) dose group (-3.1 - -13.5%) is less severe, than the maternal body weight effects at 1200 ppm (= 168 mg/kg bw) dose group (-14.9 - -20.3%) and more severe in the 1200 ppm (227 mg/kg bw) dose group(-3.1 - -36.1%), but also the substance intake was highest in F2 pups of the 1200 ppm dose group. Even, when comparing the measured substance intakes between the first and the second generation of this study, it is evident, that F1 parents during premating and F2 pups had considerably higher substance intakes compared to the respective values of the F0/F1 generation: top dose F0 females had a compound intake of 118.9 mg/kg bw during premating, while the respective compound intake of F1 females was 159 mg/kg bw.

When doing benchmark dose (BMD) calculations of the maternal and offspring PODs (points of departures) for body weight effects at lactation day 21, the female (F1) $BMDL_{05}$ is 25.5-45.3 mg/kg bw (based measured substance intake data during different phases of lactation) and the pup (F2) $BMDL_{05}$ is 39.8 mg/kg bw (using the same modelling assumptions) (see Table 17; Dammann M (2015)). This further demonstrates, that the no adverse effect levels (= PODs) between dams and pups is essentially comparable, when more accurate doses are used for estimation.

Table 17: BMD calculations for dam body weight on lactation day 0, 1, 7, 14 and 21 and pup body weight on day 21

Endpoint	**Model**	**BMD**	**BMDL**
Body weight on lactation day 1	Exponential (M2)	33.4	28.6
Body weight on lactation day 4	Exponential (M2)	33.1	28.4
Body weight on lactation day 7	Exponential (M4)	39.7	25.5
Body weight on lactation day 14	Linear	46.9	41.5

Body weight on lactation day 21*	Linear	54.7	45.3
Pup body weight on day 21	Linear	43.6	39.8

Exponential (M2) Y[dose] = a * exp{sign * b * dose}
Exponential(M4) Y[dose] = a * [c-(c-1) * exp{-b * dose}]
Linear Y[dose] = beta_0 + beta_1*dose
*BMD calculation uses mean intakes between LD 1-14

Therefore, the apparently more severe effects in pup body weights compared to parental body weight effects on PND 14 and PND 21 are considered to be linked to the higher compound intake of pups and not to a higher susceptibility. Further, any amount of the compound occurring in milk is contributing to the overall dose of the pups and this is not even included in the above assumptions.

The absence of effects on pup weights at birth in both the F1 and F2 generation pups of the two-generation study and also in the F1 pups of the one-generation study (see Table above Anonymous 2001b, 2000/1016870) is consistent with the absence of effects on fetal body weight in the dimoxystrobin rat developmental study (Anonymous 1999 a, 1999/11680), which had a higher mg/kg bw/day dose (up to 300 mg/kg bw/day) than did the reproductive toxicity studies (summaries of these studies can be found above).

Additionally, reduced serum iron levels and anemia – which is a general toxicological feature in rats treated with dimoxystrobin - as observed in dimoxystrobin-treated animals (see study summary of the modified one-generation study with dimoxystrobin (Anonymous 2001b, 2000/1016870) above is assumed to lead to reduced iron levels in milk of treated-dams, which contributes to impaired body weight development in pups (Roth et al., (1987), and Roth and Smith, (1988)).

Dosing of dams	Maternal body weight	Pups body weight (Birth and lactation)	Anemia (HGB, MCV, RBC count)	Fe levels in plasma	Fe levels in milk	Histopathology of offspring
2 g $NaNO_2$/L	GD18: - LD20: ↓	At birth: no effect ↓ (-5 - -25%) ≥LD6	HGB, MCV, RBC ↓ (LD9 + 16)*	Dams: ↓ (-56%) Offspring: ↓ (-56 - -61%) LD 15	↓ (-35% compared to controls)	Enlarged hearts, small spleen, decreased hematopoiesis of spleen, centrilobular vacuolization of liver
3 g $NaNO_2$/L	GD18: - LD20: ↓	↓ (-10 - -50%) ≥LD3	HGB, MCV, RBC ↓ (LD9 + 16)*	n.d.	n.d.	n.d.

*NaNO2 could not detected in plasma of offspring, but was present in plasma of dams

Additionally in these two publications it has been shown, that iron supplementation can reverse the sodium nitrite induced anemia and secondly, that effects on offspring body weights only occur in pups weaned by treated dams and not by pups, which were only treated via in-utero exposure (cross-fostering experiment in Roth et al., 1987). A summary of the hematological parameters in the modified one-generation toxicity study (Anonymous 2001b, 2000/1016870) is given in the Table below:

Table 18: Summary of hematological parameters in the modified one-generation toxicity study

Dose [ppm]		**Hematological parameters**						
		RBC (TERA/L)	**HGB (MMOL/L)**	**HCT (L/L)**	**MCV (FL)**	**MCH (FMOL)**	**MCHC (MMOL/L)**	**PLT (GIGA/L)**
Males, day 29	0	7.71	9.3	0.411	53.3	1.21	22.69	819
	150	7.49	9.1	0.399	53.4	1.22	22.79	800
	500	7.87	9.1	0.404	**51.4****	**1.16****	22.47	856
	1200	**8.2****	**8.5*****	0.387	**47.2*****	**1.04*****	**22.04****	896
Males, day 98								

0	8.84	9.5	0.484	54.7	1.08	19.68	709
150	8.6	9.5	0.474	55.2	1.11	**20.09****	700
500	9	9.6	0.489	54.3	1.06	19.55	706
1200	8.97	9.4	0.479	53.4	1.05	19.63	723
Females, day 29							
0	7.68	9.4	0.409	53.3	1.22	22.94	740
150	7.58	9.3	0.403	53.1	1.22	22.59	768
500	7.72	9.2	0.403	52.2	1.19	22.78	728
1200	7.96	9	0.405	51	**1.14****	**22.28****	841
Females, day 100							
0	8.5	10.4	0.498	58.8	1.22	20.76	779
150	8.31	**9.5****	0.471	56.7	**1.14***	**20.07***	763
500	9.09	10	0.505	55.7	**1.11***	**19.90*****	793
1200	**9.54***	**9.4*****	0.491	**52.0***	**1.00****	**19.21*****	**981****
Male pups, PND 21							
0	4.64	5.4	0.305	65.9	1.17	17.67	825
150	4.67	5.1	0.286	61.4**	1.08	17.62	821
500	**4.28***	**3.9*****	**0.223*****	**52.0*****	**0.91*****	17.48	1065
1200	**3.10*****	**3.0*****	**0.152*****	**47.1*****	**0.97****	**20.59***	**1227****
Female pups, PND 21							
0	4.47	5.1	0.281	63	1.14	18.08	759
150	4.78	5.1	0.291	61	1.07	17.6	690
500	4.37	**4.0*****	**0.230****	**52.3*****	**0.91*****	17.39	1084
1200	**3.02****	**2.7*****	**0.139****	**44.5*****	**0.91*****	20.65	**1471***

*p ≤ 0.05; ** p ≤ 0.02; *** p ≤ 0.002

No NOAEL was identified in this mechanistic study, however in the more recently conducted second enhanced one-generation toxicity study (Anonymous 2011a, 2011/1211676; see Table above), no effects on hematological parameters or on serum iron levels were identified up to a dose of 4 mg/kg bw. Dimoxystrobin causes anemia in subchronic and chronic studies, which is characterized by reduced blood haemoglobin (HGB), reduced mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV), indicative of an iron-deficient microcytic hypochromic anemia. The correlation of anemia and reduced iron levels was clearly shown in a rat 5-week feeding study dosed with 4500 ppm dimoxystrobin (Anonymous 2002a, 2002/1005354), where severe anemia was preceded by decreased serum iron levels detected **already 24 hours after start of treatment**. After cessation of treatment iron levels increased above control levels and a considerable, however not complete recovery of anemia was noted (see Tables below).

Table 19: Serum iron levels in a 5-week feeding study in rats (4500 ppm)

		Serum iron concentration (µmol/L)								
Dose [ppm]		**5 d before admin**	**Admin for 1 d**	**Admin for 2 d**	**Admin for 5 d**	**Admin for 15 d**	**Admin for 30 d**	**admin for 36 d**	**Admin for 19 d + 11 d recovery**	**Admin for 19 d + 17 d recovery**
Control	Mean	42.2	52.8	46.8	57.3	45.9	37.2	43.0	37.2	43.0
4500	Mean	48.5	**25.6****	**25.4****	**14.7****	**16.2****	**24.4***	**13.4****	55.9	54.9
	% dev.	+15	-52	-46	-74	-65	-34	-69	+50	+28

*p ≤ 0.05; ** p ≤ 0.01

Table 20: Hematological parameters in a 5-week feeding study in rats (4500 ppm)

		Hematological examinations			
Dose [ppm]		**HGB (mmol/L)**	**MCV (fl)**	**MCH (fmol)**	**MCHC (mmol/L)**
Control (30 d)	Mean	9.4	56.3	1.17	20.79

4500 (30 d)	Mean	**7.6****	**44.3****	**0.89****	**20.02****
	% dev.	-19.1	-21.3	-23.9	-3.7
4500 (19 d + 11 d recovery)	Mean	**8.7***	**48.7****	**1.01****	20.67
	% dev	-7.4	-13.5	-13.7	-0.6

*$p \leq 0.05$; ** $p \leq 0.01$

The overall NOAEL for serum iron decrease, which was induced in adult and in young rats (Anonymous 2005a, 2005/1004845) and changes in hematological parameters was 4 mg/kg bw (Anonymous 2002a, 2002/1014245, Anonymous 2010a, 2010/1026748, Anonymous 2011 a, 2011/1211676), thus there is no evidence for higher toxicity in young animals vs. old animals.

An overview over the most obvious necropsy observations in F1 and F2 pups, considered to be treatment-related, is given in Table 21.

Table 21: Incidence of gross necropsy observations in F1 and F2 pups

Dose [mg/kg]	0	50	150	500	1200
	F_1 pups				
Litters evaluated	23	23	25	21	24
Pups evaluated	263	250	286	255	278
- Live	261	243	281	252	261
- Stillborn	2	7	5	3	17
Milky fluid in abdomen	0	0	0	0	3 (2)
Hypoplasia of thymus	0	0	0	0	30 (13**)
Cardiomegaly	0	0	2 (1)	5 (3)	56 (19**)
Liver: pale-yellowish	0	0	2 (1)	6 (3)	42 (15**)
Total pup necropsy observations	9 (8)	12 (8)	11 (8)	19 (9)	67 (20**)
- % affected pups/litter	4.3	4.4	3.5	7.5	23.9**
	F_2 pups				
Litters evaluated	23	20	22	25	22
Pups evaluated	338	270	326	307	256
- Live	334	261	318	303	249
- Stillborn	4	9	8	4	7
Milky fluid in abdomen	0	0	0	0	8 (1)
Hypoplasia of thymus	0	0	0	0	6 (3)
Cardiomegaly	0	0	0	7 (5*)	52 (12**)
Liver: pale-yellowish	0	0	1 (1)	1 (1)	41 (9**)
Total pup necropsy observations	10 (9)	6 (5)	19 (9)	15 (10)	70 (16*)
- % affected pups/litter	2.9	2.8	5.7	4.9	25.5**

* $p \leq 0.05$, ** $p \leq 0.01$ (Wilcoxon-test, one-sided)
( ) values in brackets give litter incidence

The above mentioned findings on liver and heart are considered to be a consequence of an iron-deficiency microcytic hypochromic anemia in offspring (Cluzeaud et al., 1981;; Tanne et al. 1994; Crowe et al., 1995; Rothenbacher and Sherman, 1980; Roth and Smith, 1988), which was seen in several other repeated-dose toxicity studies with dimoxystrobin and furthermore in the modified one-generation reproduction toxicity study in Wistar rats. The milky fluids reported in the abdomen and the breast cavity are considered to be secondary to the heart-insufficiency (cardiomegaly) induced by the chronic microcytic anemia. The same effects are also seen in the F2 pups at PND21 only. Hypoplasia of thymus at the high dose is related to decreased body weights and reduced thymus weights. This is thereby not a developmental effect, as the body weight effects are induced via lactation and not via in-utero exposure.

Cardiomegaly was reported in the PND21 pups only and was not seen in PND4 pups macroscopically. Furthermore, parental F1 animals did not show cardiomegaly or other effects on the heart [see Table 21]. There is information in the literature indicating that young animals undergo cardiac remodelling secondary to anemia (Cluzeaud et al., 1981;; Tanne et al., 1994;; Crowe et al., 1995;). This is further supported by the fact, that no findings in the hearts of the offspring were seen in the rat prenatal developmental toxicity study (see Anonymous 1999 a, 1999/11680). Thus, cardiomegaly is a **transient** effect, occurring during lactation in correlation to the anemia present in offspring animals and it is **not** an irreversible structural malformation. It is not developing via in-utero exposure to dimoxystrobin. With regard to classification and labelling, this effect is clearly induced via lactational exposure to dimoxystrobin (also mechanistically supported by the cross-fostering study; Roth et al., 1987,) and thereby not to be classified for developmental toxicity

Table 22: Overview on cardiac effects in the 2-generation toxicity study

Dose [ppm]	**50**	**150**	**500**	**1200**
F0 parental	No effect	No effect	No effect	No effect
F1 pups	No effect	No effect	**Cardiomegaly** (only in PND21 pups, not PND4)	**Cardiomegaly** (only in PND21 pups, not PND4)
F1 parental	No effect	No effect	Heart dilation in 1 male animal*	No effect
F2 pups	No effect	No effect	**Cardiomegaly** (only in PND21 pups, not PND4)	**Cardiomegaly** (only in PND21 pups, not PND4)

*incidental finding due to single occurrence and no dose-relationship

In conclusion, effects seen in the reproductive toxicity studies with dimoxystrobin were direct effects of dimoxystrobin exposure during lactation and more pronounced after the start of self-feeding of the offspring animals (pup body weights, anemia, and cardiomegaly, which occurs secondary to offspring anemia). As the effects occur via lactational exposure only, no classification for developmental toxicity is justified. This is further confirmed by the absence of any effects in the offspring of the prenatal toxicity rat study. Moreover, no higher susceptibility in young animals could be detected with regard to effects on pup body weights (actual doses – being the more important parameter than dietary concentrations -, at which maternal and offspring effects occur are comparable) and on serum iron levels and anemia (clear NOAELs detected for young and adult animals).

10.10.6 Comparison with the CLP criteria

Dimoxystrobin has currently a harmonised classification for developmental toxicity Repro. Cat. 2, H361d. The observed effects on body weights, hearts (cardiomegaly) and blood (anemia) in the offspring of the reproduction toxicity studies constituted the basis for the agreed decision on this classification. Furthermore, a higher susceptibility of the offspring compared to the adults was assumed. A detailed evaluation on the findings in the reproduction toxicity studies with dimoxystrobin with a special focus on the effects considered for classification are summarized below showing that the classification with Repr. Cat. 2 (H361d) is not justified.

A comparison with the CLP criteria (Guidance to Regulation (EC) No 1272/2008; 2013) is made to show, that dimoxystrobin is not to be classified with Repr. Cat. 2, H361d. According to the CLP Classification criteria (3.7.2.2) Repr. Cat. 2 is defined as:

“Suspected human reproductive toxicant.

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.“ (Annex I: 3.7.2.1.1)

No evidence for developmental toxicity was evident from the rat developmental toxicity study, especially no effects were seen on fetal body weights or the heart of the fetuses. The two rabbit developmental toxicity

studies showed excessive maternal toxicity at 75 and 100 mg/kg bw with 2/25 and 1/25 deaths, respectively, reduced food consumption and body weight gain and disturbances of the intestinal function / diarrhea. The increased incidences of post-implantation losses and mean numbers of resorptions, and the abortion which only occurred at 100 mg/kg bw, are therefore not considered to be indicative of a specific developmental effect. Increased incidences of fused sternebra (frequently occurring variation) and severely fused sternebra – bony plate occurred in both studies, but were well within historical controls and are therefore not considered to be classifiable for developmental toxicity.

The increased number of stillborn pups in the F0/F1 generation and the increased number of dead pups in the F1/F2 generation of the 2-generation toxicity study in rats (as discussed in Chapter 10.10.2) were all very well within the historical control range of this very fertile Wistar rat strain (bred by Thomae) and are not relevant for developmental toxicity classification. The following main adverse effects of dimoxystrobin were identified and considered as critical for the classification decision in the past:

1) Microcytic hypochromic anemia
2) Reduced body weights of the offspring
3) Cardiomegaly in the offspring

Dimoxystrobin reduces the iron uptake and thus leads to the development of an iron deficiency microcytic hypochromic anemia in both parental and offspring animals, with no higher susceptibility of the offspring. This is its general toxic effect and not a specific developmental effect. Cardiomegaly is a transient effect in offspring weaned by dams treated with dimoxystrobin, which causes iron level decreases and anemia. As it is not occurring via in-utero exposure, but only after lactation, a classification for developmental toxicity is not justified.

It is known that reduced availability of iron leads to depressed body weight development in growing animals. Transient cardiomegaly is a consequence of hypoxia caused by the anemia in pups. Therefore, the effects on body weights and the heart are observed only in the presence of other toxic effects (iron deficiency/anemia). These effects are considered non-specific, secondary consequences of the anemia.

Thus, a classification for developmental toxicity is not warranted.

10.10.7 Adverse effects on or via lactation

Table 23: Summary table of animal studies on effects on or via lactation

No specific study available

Table 24: Summary table of human data on effects on or via lactation

No human data available.

Table 25: Summary table of other studies relevant for effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Two-generation reproductive toxicity study OECD 416 (Draft 1996); 87/302/EEC Part B, L 133; EPA OPPTS 870.3800; JMAFF	Test substance: Dimoxystrobin, purity: 98.4% (equivalent to substance in Chapter 1.1) See above (Table 16)	**See above (Table 16)**	Anonymous, 2001 2000/1016869 and Dammann M (2015)
Modified one-generation repro-ductive toxicity study OECD 415 (1983); 87/302/EEC Part B, L 133	Test substance: Dimoxystrobin, purity: 98.4% (equivalent to substance in Chapter 1.1) See above Table)	**See above (Table 16)**	Anonymous, 2001 2000/1016870
Mechanistic study (no guideline available) See above (Table)	Test substance: Dimoxystrobin, purity: 98.4% (equivalent to substance in Chapter 1.1) See above (Table)	**See above (Table)**	Anonymous, 2002 2002/1005354
Mechanistic study (no guideline available) See above (Table)	Test substance: Dimoxystrobin, purity: 98.4% (equivalent to substance in Chapter 1.1) See above (Table)	**See above (Table)**	Anonymous, 2005 2005/1004845
Metabolism and Distribution in	Test substance: radio-labelled	**288 ppm (10.3 mg/kg bw/day):**	Anonymous, 2001

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Domestic Animals similar to OECD 503; EPA OPPTS 860.1300 Deviations: none GLP: yes Species/strain: lactating crossbred goat Sex: female No. animals/dose: 1	(phenyl ring) dimoxystrobin, radiochemical purity: 98%; specific activity: 128.1 µCi/mg unlabelled dimoxystrobin, purity: 99.9% Dose/concentrations: 12.6, 288 ppm Route of administration: oral, gavage of capsules Duration of treatment: 5 days Frequency of treatment: daily Vehicle: none Control: none	total recovery: 93% excreta (faeces and urine): 87% amount in edible tissues: 0.2% milk: 0.1% of administered dose (total radioactive residue: 0.03 - 0.05 mg/kg) **12.6 ppm (0.5 mg/kg bw/day):** total recovery: 104% excreta (faeces and urine): 95% amount in edible tissues: 0.3% milk: <0.05% of administered dose (total radioactive residue: 0.002 - 0.003 mg/kg) **Conclusion: No accumulation of dimoxystrobin or its metabolites in the milk**	2001/5002332
Metabolism and Distribution in Domestic Animals OECD 503; 91/414/EEC 7030/VI/95 Rev. 3 (22/7/97) Appendix F; EPA OPPTS 860.1300 Deviations: none GLP: yes Species/strain: lactating Saanen cross Toggenburg goat Sex: female No. animals/dose: 1	Test substance: radio-labelled (benzyl ring) dimoxystrobin, radiochemical purity: 98.2%; specific activity: 7.6 MBq/mg unlabelled dimoxystrobin, purity: 99.9% Dose/concentrations: 11.8 ppm Route of administration: oral, gavage of gelatine capsules Duration of treatment: 7 days Frequency of treatment: daily Vehicle: removed	**11.8 ppm (0.19 mg/kg bw/day):** total recovery: 93.9% excreta (faeces and urine): 82% amount in edible tissues: < 1% milk: < 0.1% of administered dose (total radioactive residue: 0.002 mg/kg) **Conclusion: No accumulation of dimoxystrobin or its metabolites in the milk**	Anonymous, 2015 2015/1001730 and 2015/1125782

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
	prior dosing Control: none		
Livestock feeding study (residue transfer study) 91/414/EEC 7031/VI/95 Rev. 4 (22/7/97) Appendix G Deviations: none GLP: yes Species/strain: Holstein dairy cow Sex: female No. animals/dose: 3-5	Test substance: Dimoxystrobin, purity: 98.8% (equivalent to substance in Chapter 1.1) Dose/concentrations: 0, 2.5, 7.5, 25 ppm, corresponding to x1, x3 and x10 dietary burden) Route of administration: oral, gavage of gelatine capsules Duration of treatment: 30 days Frequency of treatment: daily Vehicle: none Control: yes, empty gelatine capsules	**25 ppm (0.64 mg/kg bw/day):** milk (total radioactive residue): < LOQ: 0.01 mg/kg **7.5 ppm (0.18 mg/kg bw/day):** milk (total radioactive residue): < LOQ: 0.01 mg/kg **2.5 ppm (0.07 mg/kg bw/day):** milk: not analysed, since no detectable amount was found at higher concentrations **Conclusion: No accumulation of dimoxystrobin or its metabolites in the milk**	Anonymous, 2001 2000/5259
Public literature (no guideline study) Deviations: not applicable GLP: no Species/strain: Long-Evans hooded rats Sex: female No. animals/dose: 5 - 12 females Maternally mediated, nitrite-associated iron-deficiency in offspring rats; including a cross-fostering	Test substance: Sodium nitrite Dose/concentrations: Experiment I: 0; 2 g $NaNO_2$/L; 3 g $NaNO_2$/L Experiment II: 0; 0.5 $NaNO_2$/L; 1 g $NaNO_2$/L; 2 g $NaNO_2$/L Experiment III (cross-fostering): 0, 2 g $NaNO_2$/L 4 cross-fostering groups were built: A-untreated during gestation, weaning of untreated dams B-treated during gestation, weaning of	**Experiment I** Dams: GD18: No significant effects in body weight. LD20 (≥ 2 g $NaNO_2$/L) body weight↓ Offspring: At birth: No significant differences in litter size, sex ratio, mean pup weights Lactation: Offspring body weight gain↓ (starting LD 3 in 3 g $NaNO_2$/L group and LD 6 in 3 g $NaNO_2$/L group) Mortality↑ at LD 21 (10% in 2 g $NaNO_2$/L and 30% in 3 g $NaNO_2$/L group) Hematology: LD 9 and 16: Hemoglobin↓, RBC counts↓, MCV↓ (≥ 2 g $NaNO_2$/L); sodium nitrite was not detected in plasma of LD9 and 16 pups **Experiment II** Dams: GD18 and LD20: No significant effects in body weight. Offspring	Roth et al., 1987

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
experiment Parameters: body weight, litter size, sex ration, body weight gain, hematology (Hemoglobin, RBC, MCV)	untreated dams C-untreated during gestation, weaning of treated dams D-treated during gestation, weaning of treated dams	At birth: No significant differences in litter size, sex ratio, mean pup weights Lactation: Offspring body weight gain↓ (starting LD 10 in 2 g $NaNO_2$/L group; statistically significant at LD 17) Hematology: MCV↓ (≥ 0.5 g $NaNO_2$/L; ≥ LD7); Hemoglobin↓ (2 g $NaNO_2$/L; ≥ LD 7); RBC counts↓ (2 g $NaNO_2$/L; ≥ LD 9) Experiment III Offspring: At birth: No significant differences in litter size, sex ratio, mean pup weights Cross-fostering: Group B (treated-untreated): No effect of body weight during lactation compared to Group A; no signs of anemia Group C (untreated-treated): Effect on body weight ≥ LD11; Hemoglobin↓, RBC counts↓, MCV↓ at ≥ LD14 Group D (treated-treated): Effect on body weight ≥ LD5; Hemoglobin↓, RBC counts↓, MCV↓ at ≥ LD14	
Public literature (no guideline study) Deviations: not applicable GLP: no Species/strain: Long-Evans hooded rats Sex: female No. animals/dose: 6 - 13 females Maternally mediated, nitrite-associated iron-deficiency in neo-natal rats	Test substance: Sodium nitrite Dose/concentrations: Experiment I (iron replacement): 0; 0 + Fe (to pups only); 3 g $NaNO_2$/L; 3 g $NaNO_2$/L + Fe (to pups only) Experiment II (maternal iron status): 0; 2 g $NaNO_2$/L Experiment III (pup iron status): 0; 2 g $NaNO_2$/L Route of administration: oral, drinking water Duration of	**Experiment I (iron replacement)** **3 g $NaNO_2$/L:** maternal toxicity: reduced water consumption, slightly reduced (not significant) body weight gain pup toxicity: reduced weight at birth and poor body weight development, high mortality rate, severe microcytic anaemia (by PND 14), decreased erythro-poiesis and hypoxic liver damage **3 g $NaNO_2$/L + Fe:** pup toxicity: reduced body weight development **Experiment II & III (iron status)** **2 g $NaNO_2$/L:** maternal toxicity: reduced water consumption, reduced body weight gain; serum iron level reduced (by 53%) and total iron binding capacity (TIBC) elevated (by 24%), enlarged relative heart and spleen weight, **reduced iron level (by 35%) and**	Roth and Smith, 1988

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
	treatment: gestation day 0 and lactation period (until LD 15 (Exp. II and III) - 21 (Exp. I)) Frequency of treatment: daily Vehicle: tap water Control: yes, tap water	**elevated copper level (by 24%) of the milk** pup toxicity: reduced weight at birth and poor body weight development, reduced red blood cell parameter, serum iron level reduced (by 61%) and TIBC elevated (by 35%), enlarged relative heart and reduced spleen weight, reduced liver iron content and increased liver cupper content **2 g $NaNO_3$/L - unbred:** female toxicity: reduced water consumption, reduced body weight, haematology not affected, iron status comparable to unbred control and treated lactating animals but much lower as untreated lactating animals **Conclusion: Anaemic mothers are unable to transfer sufficient iron to offspring via the milk**	
Public literature (no guideline study) Deviations: not applicable GLP: no Species/strain: Sprague Dawley rats Sex: female No. animals/dose: 5 - 8 females Effect on maternal iron nutrition during lactation on milk iron and rat neonatal iron status	Test substance: Iron Dose/concentrations: high iron (2500 ppm) diet, iron-containing (250 ppm) diet, iron-deficient (0 ppm) diet Route of administration: oral, dietary Duration of treatment: from LD 3 until LD 19 Frequency of treatment: daily Vehicle: none Control: yes, iron containing (250 ppm) diet	**high iron diet (2500 ppm)** maternal toxicity: high iron milk content but reduced during lactation, liver and plasma ferritin increased during lactation, increased HGB during lactation, high plasma iron level, low total iron binding capacity (TIBC) that decreased during lactation pup toxicity: high liver and plasma ferritin that decreased during lactation, plasma iron level increased during lactation, low TIBC increased during lactation **iron-containing control diet (250 ppm)** female (unbred) findings: no findings maternal findings: iron milk content reduction during lactation, liver and plasma ferritin decreased as compared with non-lactating females on PND 4 but increased during lactation, slightly lower HGB as compared with non-lactating females and increased HGB during lactation, plasma iron increased during lactation, TIBC higher in lactating dams but decreasing during lactation pup findings: liver ferritin decreased during lactation, HGB decreased during lactation, plasma iron level increased during lactation, TIBC increased during lactation	Anaokar, 1981

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		iron-deficient diet (0 ppm) maternal toxicity: **low iron milk content** decreased during lactation, liver and plasma ferritin decreased during lactation, decrease of HGB during lactation, low plasma iron with a decrease during lactation, high TIBC that increased during lactation pup toxicity: low liver and plasma ferritin decreased during lactation, low HGB that decreased during lactation, plasma iron level decreased during lactation, high TIBC increased during lactation **Conclusion: Direct relationship between iron intake during lactation and milk iron level, milk iron level reduction and the duration of lactation and between milk iron level and neonatal iron status**	

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

Dimoxystrobin caused decreased body weight developments in pups and dams in the reproductive toxicity studies. The effects only develop over time, being most pronounced at PND 14 and 21 after the start of self-feeding, however also detectable at days 4 and 7 (see Table below).

Table 26: Maternal (F0) and pup (F1) body weights during lactation

F0 maternal	**ppm**				
day	**0**	**50**	**150**	**500**	**1200**
1	298.5	301.7	300.9	277.6* (-7.0)	278.9* (-6.6)
4	315.3	309	312.8	294.4** (-6.6)	287.2** (-8.9)
7	323.6	320.6	322.1	308.9 (-4.5)	299.2** (-7.5)
14	334.9	333.6	332	315.2* (-5.9)	305.2** (-8.9)
21	325.7	327.3	324	312.7 (-4.0)	307.7* (-5.5)
F1 litters	**ppm**				
day	**0**	**50**	**150**	**500**	**1200**
1	6.4	6.4	6.4	6.2 (-3.1)	6.1 (-4.7)

4 preculling	9.2	9.1	9.3	8.2 (-10.9)	8.1* (-12.0)
4 postculling	9.3	9.2	9.3	8.2* (-11.8)	8.1* (-12.9)
7	14.9	14.8	15.1	13.1* (-12.1)	11.9** (-20.1)
14	32	31.8	31.7	27.7** (-13.4)	23.4** (-26.9)
21	52.6	52.9	51.8	43.8** (-16.7)	34.0** (-35.4)

*$p \leq 0.05$; ** $p \leq 0.01$
In brackets (% vs. control)

As the body weight effects only occur during lactation (increasing in severity, when pups start self-feeding) and are not evident at birth (which is also confirmed by the absence of any effect in offspring of the rat developmental toxicity study), this developmental effect during pregnancy can be excluded. There is no data on dimoxystrobin contents in rat milk, however from livestock studies (see Table 25) there is no evidence for considerable amounts of dimoxystrobin or metabolite contents in milk (especially at lower concentrations). In lactating goats after 5 consecutive daily oral administration of ^{14}C-dimoxystrobin, test item was rapidly absorbed and almost completely excreted. There was no indication of accumulation of ^{14}C-dimoxystrobin in goat milk. The parent compound was detected in milk at maximum levels of 0.1% of administered doses of 288 ppm (10.3 mg/kg bw). At lower concentrations, the radioactive residue in milk was even below 0.1% (7-day study in lactating goats that received up to 11.8 ppm 14C-dimoxystrobin in feed (0.19 mg/kg bw)). In a livestock feeding study, lactating cows were dosed with up to 25 ppm dimoxystrobin for 30 days (up to 0.64 mg/kg bw) and detected residues of 14C-dimoxystrobin were below the LOQ of 0.010 ppm.

Iron-deficient anemia is a general feature of dimoxystrobin-related toxicity. This effect is seen in young and adult rats quickly after repeated exposure, it is reversible with cessation of treatment and has clear NOAELs and there is no evidence of a higher susceptibility of young vs adult rats. Offspring of dams treated with dimoxystrobin during gestation and lactation showed decreased body weight gain and transient cardiomegalies, however these are effects occurring via lactational and/or direct exposure to diet. Milk is generally a poor source of iron. In addition, according to published literature milk of dams suffering from iron deficiency anemia contains less iron than usual (e.g. 34 µg/g dry wt in controls vs. 22 µg/g dry wt in treated dams; Roth and Smith, 1988), so that these dams are even less able to transfer sufficient iron to the young via the milk in the early postpartum period (Anaokar, 1981).

Compared to adult animals, that can store excessive amounts of iron in tissues in either two forms, ferritin or hemosiderin, which can be mobilized in an iron deficient state, pups have physiologically only very small iron stores. (Roth and Smith, 1988). The iron-deficient status of the pups seen at doses ≥ 150 ppm in the dimoxystrobin study might also have contributed to the impaired body weight development, as discussed above.

For haematological findings and reduced serum iron levels, clear NOAELs have been determined in the enhanced one-generation study that was performed at lower dose levels. No treatment-related, adverse changes of hematological parameters as well as transferrin and iron levels were observed in the parental animals and pups up to the highest dose tested (50 ppm; about 4 mg/kg bw/day). There is no evidence, that young animals are more sensitive than adults (with regard to anemia and serum iron levels). Cardiomegalies are specifically occurring in the growing animals, when the offspring is weaned by dams treated with dimoxystrobin, inducing decreased serum iron levels and anemia, however the effect is transient and not to be considered with regard to classification for developmental toxicity, but for lactation. A further mechanistic study confirmed a clear NOAEL of roughly 4 mg/kg bw/day for serum iron levels in 3-week old rats (Anonymous 2010a, 2010/1026748). Furthermore, the haematological and biochemical findings and reduced serum iron levels were shown to be reversible with no full recovery of the hematological findings (Anonymous 2002a, 2002/1005354). From the new studies it is confirmed, that there is no evidence that young rats were more sensitive towards iron related toxicity compared to adult rats (Anonymous 2005a, 2005/1004845). An overall NOAEL of 4 mg/kg bw for effects on serum iron levels has been set during the European Peer Review.

10.10.9 Comparison with the CLP criteria

Substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned based on:

(a) human evidence indicating a hazard to babies during the lactation period; and/or

(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or

(c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

No human data are available for dimoxystrobin with regard to potential hazards via lactation. In ADME / residue studies on lactating ruminants (dosed up to 25 ppm), no detectable levels of dimoxystrobin in milk were determined. In rat reproduction studies, which were dosed up to 1200 ppm, no analytical determination of the rat milk had been conducted. Based on the findings on body weight development in offspring (not evident at birth, but starting during lactation and getting more pronounced in the last phase of lactation), the presence of dimoxystrobin in milk at high doses cannot be fully excluded, which would increase the internal doses of the offspring. A lower than normal iron-content of rat milk can also not be excluded, based on the data, which could also explain the impairment of body weight development during lactation. Thus, only criterion b) of the above bulleted criteria would apply for the available data set of dimoxystrobin, as the observed body weight and cardiac effects on offspring are the consequence of an iron-deficient anaemia, occurring after direct (via feed or milk) exposure with dimoxystrobin **Thus, a classification for effects via lactation is proposed.**

10.10.10 Conclusion on classification and labelling for reproductive toxicity

In the absence of effects on sexual function or fertility in the three available generational reproductive toxicity studies. Therefore, **no classification for fertility** is proposed or is considered necessary.

There were no effects on prenatal development in rats or rabbits. The increased number of early resorptions in the first rabbit study are only seen at a severely maternally toxic dose with mortalities and no/reduced defecations. In the second rabbit study statistically significant incidences of total skeletal malformations were seen at the 75 mg/kg bw dose group, while there were no such treatment-related changes seen in the first rabbit study, dosed up to 100 mg/kg bw. The finding of total skeletal malformations is thus considered incidental. Increased incidences of fused sternebrae of high dose rabbits in both studies are within historical control data or caused by one litter with 100% affected fetuses (=1 affected pup/litter). In the reproductive toxicity studies, effects on body weights and the heart of pups are observed as a consequence of other toxic effects (iron deficiency/anemia). Also, the decreased weights (and hypoplasia) of thymus and spleen or liver discoloration are secondary effects to iron deficiency/anemia and only occurring after offspring was weaned by dams treated with dimoxystrobin and not after in-utero exposure. Statistically significantly increased number of females with stillborn pups or pups died were seen in F0 females/F1 pups, but not in F1 females/F2 pups and were within largely varying background levels; these are not considered treatment-related. Therefore, **no classification is proposed for developmental toxicity.**

In the reproductive toxicity studies, iron deficiency leading to anemia, effects on body weight and the heart of pups is considered to be also of a consequence of dimoxystrobin exposure via the milk together with the reduced iron content of the milk itself. Thus, the available data meet the criteria for classification according to Regulation (EC) No. 1272/2008 for **effects on or via lactation, with the hazard statement 'H362: May cause harm to breast-fed children'**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

DS proposed no classification of dimoxystrobin for fertility and sexual function based on the absence of treatment-related effects noted in a 2-generation reproductive toxicity study, a modified one-generation reproductive toxicity study and an enhanced one-generation reproductive toxicity study (all of them conducted according to OECD TG and GLP).

The DS proposed no classification of dimoxystrobin for development because they considered that there was no evidence for developmental toxicity in the rat and that in the developmental studies in rabbits the increased incidences of post-implantation losses and resorptions, the abortion and the increased incidences of fused sternebra and severely fused sternebra–bony plate, were considered non-specific developmental effects or the effects were within the HCD and were therefore considered not sufficient to warrant classification. The increased number of stillborn F1 pups and the increased number of dead F2 pups in the 2-generation toxicity study in rats were all within the historical control data range and were therefore considered not relevant for developmental toxicity classification. The other effects found in pups consisted of microcytic hypochromic anaemia, reduced body weights and cardiomegaly. Iron deficiency microcytic hypochromic anemia was considered not a specific developmental effect as it was occurring also in parental animals. Classification due to cardiomegaly was considered not justified because this effect was considered not to be due to in utero exposure and was considered as a secondary non-specific consequence of anemia. Also the reduced pup body weight was considered to be secondary non-specific consequence of anemia.

The DS proposed classification of dimoxystrobin for effects on or via lactation because the observed body weight and cardiac effects on offspring were considered to be consequences of an iron-deficient anaemia, occurring after direct (via feed or milk) exposure with dimoxystrobin.

Comments received during consultation

Ona manufacturer provided comments supporting the DS's proposal for removing the current harmonised classification as Repro. 2 H361d.

One MSCA considered that there were insufficient mechanistic data to clearly demonstrate that the cardiomegaly in the 2-generation study in F1 and F2 offspring was due to iron deficiency in the lactating dams. The MSCA also noted that in the developmental toxicity studies in rabbits ventricular septal defects were described suggesting that the heart might be a target for developmental toxicity. It was also considered that impairment in quality of milk due to reduction of iron levels could also be plausible and that the reported effects on pup body weight during lactation justifies classification for on or via lactation. DS replied to the MSCA in the following terms:

- Cardiomegaly was determined at pup necropsy and was seen only in F1 and F2 pups sacrificed at PND21 and in none of the F0 or F1 pups that were sacrificed or died at PND < 1 (including stillborn) or at PND 4 at any dose group. All F1 parental animals were

necropsied and the only finding related to heart was one isolated heart dilatation in one male of the 500 ppm dose group with no further effects in the heart observed in any other animals. All together, these observations, provide evidence that cardiomegaly is not an effect occurring after in utero exposure, but only after postnatal and/or lactational exposure to dimoxystrobin or to milk containing insufficient iron concentration.

- The ventral septum effects seen in rabbit development toxicity studies were clearly within historical control data ranges. The heart was a target organ in offspring rats only when young rats were directly dosed with dimoxystrobin or when offspring animals were exposed to milk with presumably lower iron content, as shown in rat generation toxicity study. In the rat prenatal developmental toxicity study with only in utero exposure, no effects on the heart were detected. It was thus concluded, that the heart was not a target organ after inutero exposure to dimoxystrobin.

Another MSCA supported no classification for fertility and sexual function but highlighted uncertainties regarding the removal of the Repr. 2 classification for adverse effects on development, especially because in the one-generation toxicity study relatively low doses were tested and because a difference in true NOAELs for anaemia between parental and offspring could not be excluded. Moreover, it was questioned whether the effects on pup body weight should be considered as direct or indirect effects (as it might affect the classification on lactation). DS replied that in the more recent enhanced one-generation toxicity study, no anaemia or decreased serum iron levels were detected in dams or offspring at the top dose of 50 ppm (the second highest dose of the original 2-generation toxicity study) and thus set the same NOAEL (50 ppm) for anaemia and serum iron level changes in adults and offspring.

One manufacturer/company provided confidential information (previously requested by EFSA during the pesticide peer review process since it was considered to be potentially relevant for classification purposes). This information contained the following items:

- Historical control data rabbit prenatal developmental toxicity studies April 1999 - November 2003;
- Historical control data rabbit prenatal developmental toxicity studies April 1997 - April 2002;
- Historical control data rabbit prenatal developmental toxicity studies May 1994 - October 2000;
- Historical control data rat prenatal developmental toxicity studies January 1994 - June 1999;
- Historical control data pup necropsy observations from reproduction toxicity studies January 2008 - December 2014;
- Benchmark Dose Calculations on body weight effects in dams and offspring of the 2-generation toxicity study;
- Benchmark Dose Calculations on body weight effects in dams and offspring of the 2-generation toxicity study (EPA BMDS Software 3.1.1);
- Graphical analysis of individual male entry into puberty (preputial separation - PPS)

data correlated with body weight (1200 ppm dimoxystrobin dose vs control);

- Graphical analysis of individual male entry into puberty (preputial separation - PPS) data correlated with body weight (500 ppm dimoxystrobin dose vs control);
- Graphical analysis of individual female entry into puberty (Vaginal opening - VO) data correlated with body weight (1200 ppm dimoxystrobin dose vs controls);
- Graphical analysis of individual female entry into puberty (Vaginal opening - VO) data correlated with body weight (500 ppm dimoxystrobin dose vs controls);
- Five different publications of the open scientific literature.

This information, in particular the historical control data was noted by RAC.

Assessment and comparison with the classification criteria

The tables below summarise the relevant animal studies with dimoxystrobin for testing both sexual function and fertility and development.

***Table**: Summary table for animal studies on adverse effects on sexual function and fertility and development with dimoxystrobin.* Only statistically ($p<0.05$)/biologically significant toxic effects are shown. m=males; f=females. HCD = Historical Control Data.

Method	Results	Reference
Two-generation reproductive toxicity study OECD TG 416 (Draft 1996) Deviations: sexual maturation data did not include the body weight at the day of criterion; anogenital distance of F2 pups was not determined; thyroid weight of the parental animals was not determined GLP: yes Wistar rats 25 animals/sex/dose Purity: 98.4% Dose/concentration: 0, 50, 150, 500, 1200 ppm Route of administration: oral in feed	**<u>Parental toxicity (F0)</u>** *1200 ppm* ↓8% (m) and ↓7% (f) body weight ↓10% bodyweight gain (m) ↓4% (m) and ↓8-13% (f) food consumption ↓6% maternal bodyweight lactation day 21 **<u>Developmental toxicity (F1 pups)</u>** *1200 ppm* 11/24 pregnant F0 with stillborn pups (46% vs 8.3% in control dams) (HCD 3-13%) 17 stillborn/335 pups delivered (5.1% vs 0.6% in control) (HCD 4-35%) ↓41% (m) and ↓40% (f) pup body weight gain days 4-21 ↓15% pup bodyweight lactation day 21 ↓6.1% (absolute) and ↑45% (relative) brain weight ↓58% (absolute) and ↓37% (relative) thymus weight ↓52% (absolute) and ↓28% (relative) spleen weight Cardiomegaly 19 % litter incidence (0 in control) Pale-yellowish liver 15% litter incidence (0 in control) Hypoplasia of thymus 13% litter incidence (0% in control) *500 ppm* ↓29% (absolute) and ↓16% (relative)	Anonymous, 2001 2000/1016869

Duration of treatment: F0 ♂: 74 days prior mating, up to 2 weeks mating period ♀: 74 days prior mating, up to 2 weeks mating period, continuously exposed during gestation up to weaning (LD 21) F1 ♂: from weaning for at least 76 days, up to 2 weeks mating period ♀: from weanling for at least 76 days, up to 2 weeks mating period, continuously exposed during gestation up to weaning (LD 21)	thymus weight ↓26% (absolute) and ↓12% (relative) spleen weight **Parental toxicity (F1)** *1200 ppm* ↓18% (m) and ↓15% (f) body weight ↓16% (m) and ↓12-26% (f) food consumption ↓35% maternal bodyweight lactation day 21 *500 ppm* ↓9% (m) and ↓5% (f) body weight ↓8% (m) and ↓4-10% (f) food consumption ↓17% maternal bodyweight lactation day 21 **Sexual function and fertility (only in F1 dams)** *1200 ppm* Delay in sexual maturation (days to criterion 40.6 vs 34.9 in control in females, 47.8 vs 43.4 in males) Implantation sites per dams: 12.9 (HCD: 11.5-18.3%) Pups per F1 dam: 11.8 (HCD: 11.1-15.0%) *500 ppm* Delay in sexual maturation (days to criterion 36.4 vs 34.9 in control in females) **Developmental toxicity (F2 pups)** *1200 ppm* ↓42% (m) and ↓43% (f) pup body weight gain days 4-21 19 pups died/260 live born pups (HCD: 4-31) Viability index day 4: 92% (97% control; HCD range 83-99%) ↓36% pup bodyweight lactation day 21 ↓8.1% (absolute) and ↑46% (relative) brain weight ↓52% (absolute) and ↓25% (relative) thymus weight ↓55% (absolute) and ↓28% (relative) spleen weight Cardiomegaly 12% litter incidence (0 in control) Pale-yellowish liver 9% litter incidence (0 in control) *500 ppm* ↓14% pup bodyweight lactation day 21 ↓18% absolute thymus weight ↓19% (absolute) and ↓8% (relative) spleen weight Cardiomegaly 5% litter incidence (0 in control)

Modified one-generation reproductive toxicity study OECD TG 415 (1983) Deviations: only 10 animals/sex/generation were used; exposure before mating was shorter than 70 days GLP: yes Wistar rats 10 animals/sex/ dose Purity: 98.4% Dose/concentration: 0, 150, 500, 1200 ppm Route of administration: oral in feed Duration of treatment: ♂: 47 days prior mating, up to 2 weeks mating period ♀: 47 days prior mating, up to 2 weeks mating period, continuously exposed during gestation up to weaning (lactation day 21)	<u>Parental toxicity</u> *1200 ppm* ↓17% bodyweight (m) (week 13) ↓24% body weight gain (m) (week 13) ↓9% bodyweight (f) (*post coitum* day 20) ↓18% bodyweight gain (f) (*post coitum* day 20) ↓9% (m) and ↓12% (f) food consumption Haematological changes Mycrocytosis *500 ppm* ↓11% bodyweight (m) (week 13) ↓14% body weight gain (m) (week 13) ↓11% (m) food consumption Haematological changes Mycrocytosis <u>Developmental toxicity</u> *1200 ppm* ↓38% bodyweight (day 21) ↑57% relative heart weight (cardiomegaly) Haematological changes Pale discoloration of liver and kidney ↓20% (f) absolute liver weight ↑15% (m) relative liver weight ↑26% (f) relative liver weight Milky fluid in abdomen and/or thorax after organ evisceration *500 ppm* Haematological changes Pale discoloration of liver and kidney Milky fluid in abdomen and/or thorax after organ evisceration <u>No treatment related effects on sexual function and fertility at any dose</u>	Anonymous, 2001b 2000/1016870
Enhanced one-generation reproductive toxicity study OECD TG 416 (2001) GLP: Yes Route of administration: oral in feed Wistar rats 25 animals/sex/group Deviations: study design was limited to 1 generation; estrus cycle	<u>No treatment related effects on sexual function and fertility or developmental or parental toxicity at any dose</u>	Anonymous, 2011 2011/1211676

and sperm parameters were not determined; organ weight determination, gross necropsy and histopathology were not included; haematology and determination of iron and transferrin was included for blood samples of parental animals taken before sacrifice as well as of pups on PND 7, 14, 21 Purity: 98.5% Dose/concentration: 0, 10, 20, 50 ppm Duration of treatment: ♂: 73 days prior mating, up to 2 weeks mating period ♀: 73 days prior mating, up to 2 weeks mating period, continuously exposed during gestation up to weaning (LD 21), however, during lactation exposure levels were reduced by 50% due to increased food consumption during that phase		

Table**: Summary table for animal studies on developmental toxicity with dimoxystrobin.** Only statistically ($p<0.05$)/biologically significant toxic effects are shown. HCD = Historical Control Data.

Method	**Results**	**Reference**
Prenatal developmental toxicity study OECD TG 414 (Draft 1996) Gavage No deviations GLP: yes Wistar rats 25 females/dose Purity: 98.8%	<u>300 mg/kg bw/day</u> ↓8% food consumption ↓10% bodyweight gain <u>120 mg/kg bw</u> ↓12% bodyweight gain <u>No developmental toxicity at any dose</u>	Anonymous, 1999 1999/11680

Dose/concentrations: 0, 60, 120, 300 mg/kg bw/day Treatment: GD 6 – 19 Vehicle: 0.5% tylose CB 30000 in doubly distilled water		
Prenatal developmental toxicity study OECD TG 414 Gavage Deviations: only the head of those foetuses that showed severe abnormal findings were subject to the histopathological examinations. GLP: yes Himalayan rabbit Purity: 98.4% Dose/concentrations: 0, 25, 50, 100 mg/kg bw/day Duration of treatment: GD 7 - 28 Vehicle: 0.5% tylose CB 30000 in doubly distilled water	<u>100 mg/kg bw/day</u> Maternal toxicity: Diarrhoea and further no defecation 1 doe found dead ↓ 15% food consumption (GD7-28) ↓ 40% bodyweight gain (GD7-28) Developmental toxicity: 1 abortion and further sacrifice 2.2 mean number of resorptions (control = 1) 2.0 mean number of early resorptions (control 0.8) Fused sternebrae litter incidence = 47% vs 8% in control (HCD = 0-50%) Fused sternebrae foetuses/litter = 15.2% vs 0.9% in control (11.1% at high dose if excluding a dam which had 100% affected foetuses (=1 affected pup)/litter) from the evaluation) (HCD = 0-13.5%) <u>50 mg/kg bw/day</u> Maternal toxicity: Diarrhoea (12 dams on day 8) and further no defecation (1 dam on day 9) ↓ 6% food consumption (GD7-28) Developmental toxicity: Fused sternebrae litter incidence = 35% Fused sternebrae foetuses/litter = 6.5% <u>25 mg/kg bw/day:</u> Maternal toxicity: Diarrhoea (2 dams on day 8) Developmental toxicity: Fused sternebrae litter incidence = 36% Fused sternebrae foetuses/litter = 5.1%	Anonymous, 2001 2001/1016351
Prenatal developmental toxicity study OECD TG 414 (2001) Gavage	<u>75 mg/kg bw/day</u> Maternal toxicity: 16/25 diarrhoea 10/25 no defecation (most of them with previous diarrhoea)	Anonymous, 2001 2001/1016351

	2 does found dead
No deviations	↓18 % food consumption (GD7-28) ↓55% body weight gain (GD7-28)
GLP: yes	
	Developmental toxicity
Himalayan rabbit	Total skeletal malformations: litter incidences: 41% (control 13%); foetuses/litter: 9.4±14
25 animals/dose	(control 2.2±6.16)
	Bony plate (sternebra severely fused-malformation): Litter incidence 14 % vs 0 % in control; foetuses/litter: 2.2±6.16 (control 0)
Purity: 98.4%	
Dose/concentrations: 0, 5, 20, 75 mg/kg bw/day	<u>20 mg/kg bw/day</u>
	Maternal toxicity:
Duration of treatment: GD 7-28	6/25 diarrhoea
Vehicle: 0.5% tylose CB 30000 in doubly distilled water	

Comparison with the criteria

<u>Sexual function and fertility</u>

In the rat 2-generation reproduction toxicity study, there were no treatment-related significant effects on sexual function or fertility. Treatment with dimoxystrobin up to the concentration of 1200 ppm had no effects on the oestrous cycle, the number, morphology and motility of sperm as well as on male or female fertility. Male and female fertility indices ranged between 80 and 100% without relation to dose. Dimoxystrobin treatment did not affect the reproductive performance as was evident from the absence of effects on the pre-coital interval or gestation lengths as well as gestation (96 to 100%) or live birth indices (95 to 99%).

A reduction on the implantation sites and pups per F1 dams were noted. However, these effects were within the historical control data range. In addition, a delay in sexual maturation of both males and females was noted in F1 generation at the highest dose. However, RAC notes that the delayed onset of puberty is likely secondary to lower offspring body weights.

The enhanced one-generation toxicity study conducted according to OECD TG 416 (2001) and the modified one-generation toxicity study conducted according to OECD TG 415 (1983) showed no effects on sexual function and fertility; although the first of these two studies was performed using very low dosing and therefore the information provided in this study is not conclusive enough. RAC agrees with the DS's proposal for **no classification of dimoxystrobin for sexual function and fertility.**

<u>Development: Prenatal toxicity studies in rats and rabbits</u>

No developmental toxicity was observed at the highest dose tested (300 mg/kg bw/day) in the prenatal toxicity study in rats. In rabbits, different effects were reported in two prenatal toxicity studies where doses of dimoxystrobin were 0, 25, 50 and 100 mg/kg bw/day in the first study and 0, 5, 20 and 75 mg/kg bw/day in the second study.

In the first study maternal toxicity was seen at 100 mg/kg bw/day, indicated by maternal mortality (1 female was found dead), diarrhoea, no defecation, 15% decrease in food

consumption and 40% decrease in body weight gain between GD7 and GD28. At 50 mg/kg bw/day, one dam had no defecation on day 9 and 12 dams had diarrhea on day 8. Transient drop in food consumption and effects on body weight were observed at all dose levels. At 100 mg/kg bw/day the significant maternal toxicity was considered to result in increased resorptions (mainly early resorptions) and post implantation loss (due to an increased number of early resorptions). As a consequence gravid uterus weights were lower at this dose level without attaining statistical significance. One dam was sacrificed due to abortion. The effects on sternebrae are assessed below.

In the second study severe maternal toxicity was seen at 75 mg/kg bw/day, indicated by maternal deaths (2 females died), diarrhoea and no defecation. Mean body weight gain during treatment was significantly decreased at the high dose of 75 mg/kg bw/day (about 55%) and non-statistically significant at the mid dose of 20 mg/kg bw/day (about 19%). At 75 mg/kg bw/day this maternal toxicity was considered to result in increased non-statistically significant resorptions (mainly early resorptions) and post implantation loss. As a consequence, gravid uterus weights were lower at this dose level but without attaining statistical significance.

Thus, increased incidences of post-implantation losses and mean numbers of resorptions co-occurred with severe maternal toxicity (including mortalities, clinical signs and reductions in body weight gain between 40 and 55%) and were therefore not considered by RAC as indicative of developmental toxicity.

The incidence of total skeletal malformations was statistically significantly increased in the second rabbit study at the 75 mg/kg bw/day dose. However, there were no treatment-related malformations seen in the first rabbit study, which had been dosed up to 100 mg/kg bw/day. In the table below, the incidences for total skeletal malformations are summarized for both rabbit studies, which clearly demonstrates the absence of a dose-response. In consequence, RAC considers the effects on total skeletal malformations at 75 mg/kg bw/day as incidental.

***Table**: Incidences of total skeletal malformations in both rabbit studies (Anonymous 2001a, 2000/1016867 and 2001/1016351).* * = p < 0.05

	Dose (mg/kg bw/day)							
Parameter	**Control 1**	**Control 2**	**5**	**20**	**25**	**50**	**75**	**100**
Fetal incidence [N (%)]	4 (2.6)	3 (1.8)	6 (3.6)	3 (2.0)	2 (1.2)	3 (1.9)	11 (8.1)	5 (4.8)
Litter incidence [N (%)]	3 (12)	3 (13)	6 (25)	2 (8.0)	2 (8.0)	3 (13)	9* (41)	4 (21)
Affected fetuses/litter (Mean ± SD) [%]	3.4± 9.9	2.2± 6.2	3.4± 6.1	1.6± 5.7	2.4± 7.5	2.4± 7.5	9.5*± 14	6.9± 17

The increased incidences of fused sternebrae was seen above the concurrent controls at all doses in the first study and at the top dose in the second study and within or slightly above the historical control data range in both studies. RAC considers that the fused sternebrae raises a concern for developmental toxicity. The finding *"severely fused sternebra (bony plate)"* was slightly but statistically significantly increased in the second study with a litter incidence of 3. This finding was also observed in the control group of the first rabbit study,

with the same incidence (3) and is therefore considered an incidental finding.

***Table**: Incidences of fused sternebra, severely fused sternebra and septum ventricular defects in the first (Anonymous, 2001, 2001/1016351) and the second (Anonymous, 2001 2001/1016351) rabbit prenatal toxicity study.* HCD = Historical control data covering a time span of roughly ± 5 years around the experimental date. [1]when excluding dam #87 (which had 100% affected foetuses (=1 affected pup)/litter) from the evaluation the incidence decreases to 11.1%. [2]comparable incidence as the control group of the first study

Finding	Control	Low dose	Mid Dose	High Dose
Fused sternebra - First study				
Fetal incidence [N (%)] (HCD 0.0-10.7)	2 (1.3)	9 (5.5)	10 (6.2)	11 (10)
Litter incidence [N (%)] (HCD 0.0-50.0%)	2 (8.0)	**9*** **(36)**	**8*** **(35)**	**9**** **(47)**
Affected fetuses/litter (Mean) [%] (HCD 0.0-13.5%)	0.9	**5.1****	**6.5****	**15.2****[1]**
Fused sternebra - Second study				
Fetal incidence [N (%)] (HCD 0.0-10.7)	5 (3.0)	8 (4.8)	3 (2.0)	16 (12)
Litter incidence [N (%)] (HCD 0.0-50.0%)	4 (17)	5 (21)	2 (8.0)	8 (36)
Affected fetuses/litter (Mean) [%] (HCD 0.0-13.5%)	2.7	4.6	2.5	11.2
Membranous ventricular septum defect – Second study (not seen in first study)				
Fetal incidence [N (%)]	1 (0.6)	0 (0.0)	1 (0.7)	3 (2.2)
Litter incidence [N (%)] (HCD: 0 – 17.6%)	1 (4.2)	0 (0.0)	1 (4.0)	3 (14)
Affected fetuses/litter (Mean) [%]	0.5	0.0	0.5	2.4
Sternebrae severely fused (bony plate) – First study				
Fetal incidence [N (%)]	3 (2.0)	0 (0.0)	0 (0.0)	1 (1.0)
Litter incidence [N (%)]	3 (12.0)	0 (0.0)	0 (0.0)	1 (5.3)
Affected fetuses/litter (Mean) [%]	2.6	0.0	0.0	1.8
Sternebrae severely fused (bony plate) – Second study				
Fetal incidence [N (%)]	0 (0.0)	0 (0.0)	1 (0.7)	3 (2.2)
Litter incidence [N (%)]	0 (0.0)	0 (0.0)	1 (4.0)	3 (14)
Affected fetuses/litter (Mean) [%]	0.0	0.0	0.5	**2.2*[2]**

Development: Developmental toxicity investigated in generation toxicity studies

Stillborn and reductions in viability

An increased litter (11 (46%) vs 2 (8.3%) in control) and foetal (17 (5.1%) vs 2 (0.6%) in control) incidence in the number of stillborn F1 pups and a decreased viability index of F2

pups between PND 0-4 (92% vs 97% in control) were reported at the top dose in the 2-generation reproduction toxicity study in rats. These incidences were within the historical control data range. However, RAC notes that the concurrent control is the most relevant control and that these effects show a dose-response with some effects at 500 ppm and no effects at 0, 50 and 150 ppm. Thus, RAC considers that effects on stillborn index and reductions in viability index raise a concern for developmental toxicity.

Pup body weight

Significant effects on body weight were essentially absent at birth in the offspring animals. Mean pup body weights of F1 pups in the 500 and 1200 ppm dose test groups were statistically significantly reduced compared to controls from PND 4 onwards. Maternal body weights were between 4 and 7% lower than controls in the 500 ppm dose group and between 6 and 9% lower in the 1200 ppm dose group. In the F1 pups the body weights were 3-17% lower than in controls at 500 ppm and 5–35% lower than in controls at 1200 ppm (the table below).

***Table**: Maternal (F0) and pup (F1) body weights during lactation in the 2-generation reproductive toxicity study (Anonymous, 2001, 2000/1016869).* * = p < 0.05; ** P < 0.01.

	ppm				
Day	**0**	**50**	**150**	**500**	**1200**
F0 MATERNAL					
1	298.5	301.7	300.9	**277.6* (-7%)**	**278.9* (-7%)**
4	315.3	309	312.8	**294.4** (-7%)**	**287.2** (-9%)**
7	323.6	320.6	322.1	308.9 (-5%)	**299.2** (-8%)**
14	334.9	333.6	332	**315.2* (-6%)**	**305.2** (-9%)**
21	325.7	327.3	324	312.7 (-4%)	**307.7* (-6%)**
F1 LITTERS					
1	6.4	6.4	6.4	6.2 (-3%)	6.1 (-5%)
4 preculling	9.2	9.1	9.3	8.2 (-11%)	**8.1* (-12%)**
4 postculling	9.3	9.2	9.3	**8.2* (-12%)**	**8.1* (-13%)**
7	14.9	14.8	15.1	13.1* (-12%)	**11.9** (-20%)**
14	32	31.8	31.7	**27.7** (-13%)**	**23.4** (-27%)**
21	52.6	52.9	51.8	**43.8** (-17%)**	**34.0** (-35%)**

A similar picture with regard to pup body weights is seen in the second generation of this 2-generation toxicity study (the table below). Body weight effects are seen in the F2 pups from 150 ppm. As in F1 generation, no effects on pup body weight were observed at birth but the effect was statistically significant from PND 7 onwards.

***Table**: Maternal (F1) and pup body weights (F2 litters) during lactation in the 2-generation reproductive toxicity study (Anonymous, 2001, 2000/1016869).* * = p < 0.05; ** P < 0.01.

	ppm				
Day	**0**	**50**	**150**	**500**	**1200**
F1 MATERNAL					
1	308.7	306.0	299.9	**275.8** **(-11%)**	**248.7** **(-20%)**
4	317.9	320.3	309.6	**288.4** **(-9%)**	**256.2** **(-19%)**
7	327	328.9	319.4	**299.5** **(-8%)**	**265.7** **(-19%)**
14	347.2	345.9	342	**314.9** **(-9%)**	**276.6** **(-20%)**
21	330	330.6	330.2	313.4	**280.7** **(-15)**
F2 LITTERS					
1	6.4	6.4	6	6.2	6.2
4 preculling	9.2	9.5	8.4	9.2	8.5
4 postculling	9.2	9.6	8.5	9.2	8.5
7	14.8	15.3	**13.2*** **(-11%)**	14.2 (-4%)	**11.8** **(-20%)**
14	31.9	32.7	**29.2*** **(-9%)**	**28.8** **(-10%)**	**22.3** **(-30%)**
21	51.2	52.3	**47.1*** **(-8%)**	**44.3** **(-14%)**	**32.7** **(-36%)**

It was expected by the DS that pups showed more severe effects on body weights on PND 14 and PND 21 since they were estimated to receive higher daily doses of dimoxystrobin compared to the dams at the same dietary doses during the last week of lactation. The dietary exposure was continuous throughout the 2-generation study (and the supplementary modified one-generation study, see below), and dietary concentrations were not reduced during lactation. A comparison of the actually measured maternal (F0 and F1) dimoxystrobin doses is shown in the table below. When comparing the measured substance intakes between the first and the second generation of this study, it is evident, that F1 parents during premating and F2 pups (during the last week of lactation) had considerably higher daily substance intakes compared to the respective values of the F0/F1 generation. The estimated daily values are considerably higher in pups during the last week of lactation compared to female adults (a dose of 227.7 mg/kg bw/day is estimated for F1 pups, while females consume only 168.2 mg/kg bw/day over the lactation period in the 1200 ppm dose group). This difference is even more pronounced in the F2 pups of the 1200 ppm dose group with an estimated daily test substance intake of 315.4 mg/kg bw/day during the last week of lactation compared to 168 mg/kg bw/day in the respective high dose females (table below).

***Table**: Approximate daily compound exposure (mg/kg bw/day) to parental (F0 and F1) animals and estimated daily exposure (mg/kg bw/day) to F1 and F2 pups during the last week of lactation (excluding amount transferred in milk) in the 2-generation study.*

	Compound exposure (ppm)				
ppm in diet	**0**	**50**	**150**	**500**	**1200**
F0 male (premating)	0	4.7	14.1	46.4	108.8
F0 female (premating)	0	5.1	15.6	49.9	118.9
F0 Female (gestation)	0	4.5	13.6	43.6	102.5

F0 Female (lactation)	0	7.6	22.1	74.5	168.2
F1 pups	0	9.8	29.7	96.3	227.7
F1 pups (not corrected)	0	6.1	17.9	59.1	135.4
F1 Male (premating)	0	5.9	18.2	61.8	156.4
F1 Female (premating)	0	6.2	18.6	63.7	159
F1 Female (gestation)	0	4.6	13.6	46.1	107.8
F1 Female (lactation)	0	7.4	22.4	75.4	168
F2 pups	0	12.1	36.8	125.5	315.4
F2 pups (not corrected)	0	6	18	60.8	138

RAC notes that a higher daily compound intake by pups is a plausible (although not conclusive) explanation for the difference in magnitude of effects between dams and pups during the last week of lactation. However, this is not considered by RAC as a justification for disregarding the effects on pup body weight. Pups were severely affected already before they started self-feeding. In addition, even though for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure, developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. These effects can be also manifested at any point in the life span of the organism (CLP Annex I: 3.7.1.4.). The effects on pup body weight were consistently reported in all generations and in all available generational studies. Thus, RAC considers that the severe reductions in pup body weight raise a concern for developmental toxicity.

Microcytic hypochromic anaemia

The modified one-generation reproductive toxicity study shows significant reductions in several haematological parameters in exposed pups as compared to controls on PND 21, especially in RBC (33 and 35%), HGB (44 and 47%) and HCT (50 and 51%) (at top dose in males and females, respectively). In parental animals the slight effects on RBC pointed to the opposite direction than in pups (i.e. the values were higher in treated parental animals as compared to controls) and the effects on the other haematological parameters were of lower severity than in pups. The daily doses after pups start self-feeding may have been higher than those in adults, but as discussed above, this is not considered by RAC as a reason for discounting the effects observed in pups. There is also no information allowing to conclude that the exposure of the pups via parental animals did not play a role in the manifestation of the effects in pups. Thus, RAC notes that the severe anaemia in pups induced by dimoxystrobin exposure raises a concern for developmental toxicity.

Cardiomegaly

Cardiomegaly was determined at pup necropsy and was seen in F1 (2-generation study and modified one-generation reproductive toxicity study) and F2 (2-generation study) pups sacrificed at PND21 and in none of the pups sacrificed or that died at PND < 1 (including stillborn pups) or at PND 4 at any dose group. All F1 parental animals were necropsied and

the only finding in heart was one isolated heart dilatation in one male at 500 ppm. No findings in the hearts of the offspring were either observed in the rat prenatal developmental toxicity study (Anonymous 1999 a, 1999/11680). There is information in the literature indicating that young animals can undergo cardiac remodelling secondary to nutritional anaemia (Tanne *et al.*, 1994) supporting the link between anaemia and cardiac effects in pups. After dimoxystrobin exposure, cardiomegaly was observed in pups only at PND21 and correlated with the severe anaemia present in offspring animals. Thus, considering that cardiomegaly is an effect of concern and even if it would be secondary to anaemia, anaemia would be considered by RAC as a specific mechanism that does not reduce the level of concern for cardiomegaly. RAC considers that cardiomegaly raises a concern for developmental toxicity.

Other gross necropsy observations

Reductions in absolute and/or relative weight of different organs (brain, thymus, spleen and liver) were reported at the top and mid dose of F1 and F2 generations of the 2-generation toxicity study and at the top dose in pups of the modified one-generation reproductive toxicity study. These effects can be partly, but not completely, explained by the reductions in the pup body weight. Anaemia can be the explanation for the organ discoloration found mainly in liver and kidney. Hypoplasia of thymus was also detected at the top dose of the F1 and F2 generation, although statistical significance was reached only in the F1 generation. This hypoplasia of thymus at the high dose may be related to anaemia, which is supported by finding in open scientific literature showing lesions in spleens and thymuses of the iron-deficient rats (Rothenbacher and Sherman, 1980). However, the mechanism(s) of the observed effects were not investigated. The milky fluids reported in the abdomen and the breast cavity of pups in the modified one-generation reproductive toxicity study are considered to be secondary to the heart-insufficiency (cardiomegaly).

Overall, RAC notes that the effects observed especially in the thymus and spleen raise concern for developmental toxicity.

Conclusion

RAC concludes that dimoxystrobin meets the CLP criteria for Repr. 2, H361d because of anaemia, cardiomegaly and severe reductions in body weight in rat pups. The co-occurring maternal toxicity was not severe and therefore the developmental effects are considered not to be secondary non-specific consequences of maternal toxicity. RAC concludes that the increased incidences in fused sternebrae in rabbits and the increased number of stillborn F1 pups and a decreased viability index of F2 pups between PND 0-4, hypoplasia of thymus and decreases in organ weights (especially in thymus and spleen) in the two-generation study in rats increase the level of concern and these effects are considered as supportive evidence for classification for developmental toxicity. Themode of action of the developmental effects have not been demonstrated, but RAC concludes that even if the effects in developing animals were secondary to anaemia, anaemia would be considered by RAC as a specific mechanism that does not reduce the concern for the adverse effects in development. As in the two-generation studies, exposure is continuous from the prenatal period, the role of exposure via the mother cannot be excluded even if effects would appear only postnatally. However, according to CLP, developmental toxicity is not limited only to effects via parental animals as it includes any effect which interferes with normal development of the conceptus resulting also from exposure of the developing offspring postnatally to the time of sexual

maturation.

RAC concludes that dimoxystrobin warrants **classification as Repr. 2, H361d.**

Lactation

There is no data on dimoxystrobin contents in rat milk, however, from livestock studies there is no evidence indicating the existence of considerable amounts of dimoxystrobin or metabolite contents in milk (especially at lower concentrations). In lactating goats after 5 consecutive daily oral administration of ^{14}C-dimoxystrobin, the test item was rapidly absorbed and almost completely excreted. There was no indication of accumulation of ^{14}C-dimoxystrobin in goat milk. The parent compound was detected in milk at maximum levels of 0.1% of administered doses of 288 ppm (10.3 mg/kg bw/day). At lower concentrations, the radioactive residue in milk was even below 0.1% (7-day study in lactating goats that received up to 11.8 ppm ^{14}C-dimoxystrobin in feed (0.19 mg/kg bw/day)). In a livestock feeding study, lactating cows were dosed with up to 25 ppm dimoxystrobin for 30 days (up to 0.64 mg/kg bw/day) and detected residues of ^{14}C-dimoxystrobin were below the limit of quantification of 0.010 ppm.

Milk is generally a poor source of iron. In addition, according to published literature, milk of dams suffering from iron deficiency anaemia contains less iron than usual (e.g. 34 µg/g dry wet in controls vs. 22 µg/g dry wet in treated dams), so that these dams are even less able to transfer sufficient iron to the young via the milk in the early postpartum period.

Substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned based on:

a) human evidence indicating a hazard to babies during the lactation period; and/or
b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or
c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

No human data is available for dimoxystrobin with regard to potential hazards via lactation. In ADME / residue studies on lactating ruminants (dosed up to 25 ppm), no detectable levels of dimoxystrobin in milk were determined. In rat reproduction studies, which were dosed up to 1200 ppm, no analytical determination of the rat milk had been conducted. Roth and Smith (1988) documented that mother rats with nitrite-induced iron deficiency produced milk of reduced iron content. However, there are no data about the iron content in milk generated by dimoxystrobin-exposed females.

In conclusion, there is no clear evidence of adverse effect in the offspring due to transfer of dimoxystrobin in the milk or adverse effect on the quality of the milk. Moreover, there is no toxicokinetic evidence indicating that the substance is present in potentially toxic levels in breast milk. Thus, overall, **RAC does not support the DS's proposal for classification of dimoxystrobin for effects on or via lactation H362 (may cause harm to breast-fed children).**

10.11 Specific target organ toxicity-single exposure

Not assessed in this CLH dossier.

10.11.1Conclusion on classification and labelling for STOT SE

Not assessed in this CLH dossier.

10.12 Specific target organ toxicity-repeated exposure

There are not specific target organ toxicity studies for dimoxystrobin, data from the following studies were used to evaluate the effects of dimoxystrobin in this respect.

Table 27: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Modified one-generation reproductive toxicity study OECD 415 (1983); 87/302/EEC Part B, L 133 Deviations: only 10 animals/sex/generation were used; exposure before mating was shorter than 70 days; haematology was included for blood samples of parental animals taken before the mating period and before sacrifice as well as of pups on PND 21 GLP: yes Species/strain: Wistar rats Sex: male/female No. animals/sex/dose: 10	Test substance: Dimoxystrobin, purity: 98.4% (equivalent to substance in Chapter 1.1) Dose/concentration: 0, 150, 500, 1200 ppm Route of administration: oral in feed Duration of treatment: ♂: 47 days prior mating, up to 2 weeks mating period ♀: 47 days prior mating, up to 2 weeks mating period, continuously exposed during gestation up to weaning (LD 21) Control: yes, plain diet	**1200 ppm (130 mg/kg bw/day):** parental toxicity: reduced food consumption, impaired body weight gain, microcytic hypochromic anaemia developmental toxicity: impaired body weight gain, microcytic hypochromic anaemia, increased relative heart weights, cardiomegaly (only in PND21 pups), yellowish liver discoloration, milky fluid in abdomen, pale kidneys (in PND21 pups only; not observed on PND 4 macroscopically) **500 ppm (57 mg/kg bw/day):** parental toxicity: reduced food consumption, impaired body weight gain, microcytic hypochromic anaemia developmental toxicity: impaired body weight gain, microcytic hypochromic anaemia, increased relative heart weights, cardiomegaly (only in PND21 pups), yellowish liver discoloration, milky fluid in abdomen, pale kidneys (in PND21 pups only; not observed on PND 4 macroscopically) **150 ppm (18 mg/kg bw/day):** parental toxicity: microcytic hypochromic anaemia (slight) developmental toxicity: microcytic hypochromic anaemia (slight), increased reticulocytes **NOAEL (Systemic toxicity (parents):** **150 ppm (8 mg/kg bw/day)**	Anonymous, 2001 2000/1016870
Two-generation reproductive toxicity study OECD 416 (Draft 1996); 87/302/EEC	Test substance: Dimoxystrobin, purity: 98.4% (equivalent to substance in Chapter	**1200 ppm (M: 109 mg/kg bw; F, premating: 119; gestation: 103; lactation: 168 mg/kg bw/day) F0 adults/F1 pups:** parental toxicity: reduced food consumption, impaired body weight and body weight gain,	Anonymous, 2001 2000/1016869 and

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Part B, L 133; EPA OPPTS 870.3800; JMAFF Deviations: sexual maturation data did not include the body weight at the day of criterion; anogenital distance of F2 pups was not determined; thyroid weight of the parental animals was not determined GLP: yes Species/strain: Wistar rats Sex: male/female No. animals/sex/ dose: 25	1.1) Dose/concentration: 0, 50, 150, 500, 1200 ppm Route of administration: oral in feed Duration of treatment: <u>F0:</u> ♂: 74 days prior mating, up to 2 weeks mating period ♀: 74 days prior mating, up to 2 weeks mating period, continuously exposed during gestation up to weaning (LD 21) <u>F1:</u> ♂: from weanling for at least 76 days, up to 2 weeks mating period ♀: from weanling for at least 76 days, up to 2 weeks mating period, continuously exposed during gestation up to weaning (LD 21) Control: yes, plain diet	increased number of females with stillborn pups <u>developmental toxicity:</u> increased no. of stillborn (within historical control range), impaired body weight and body weight gain starting at PND 4, delays in development landmarks, decreased weights of thymus and spleen, hypoplasia of thymus, yellowish liver discoloration, cardiomegaly (in PND 21 pups only at necropsy; not observed in PND4 pups macroscopically) **1200 ppm (M: 156 mg/kg bw; F premating: 159, gestation: 108; lactation 168 mg/kg bw) F1 adults/F2 pups** <u>parental toxicity</u>: reduced food consumption, impaired body weight and body weight gain <u>developmental toxicity</u>: decreased number of pups delivered / dam within historical controls, impaired body weight and body weight gain starting PND 4, delays in development landmarks considered secondary to body weight effects; decreased weights of thymus and spleen; yellowish liver discoloration and cardiomegaly (in PND 21 pups only at necropsy; not observed in PND4 pups macroscopically), hypoplasia of thymus (not statistically significant) **500 ppm (F0 M: 47 mg/kg bw; F premating: 50, gestation: 44; lactation 75 mg/kg bw; F1 M: 62 mg/kg bw; F premating: 64, gestation: 46; lactation 75 mg/kg bw) F0 and F1::** <u>parental toxicity:</u> reduced food consumption, impaired body weight and body weight gain <u>developmental toxicity:</u> reduced no. of live born, increased no. of stillborn, reduced no. of implants, reduced no. of delivered pups (findings inconsistent between both generations), impaired body weight and body weight gain, delays in development landmarks, decreased weights of thymus and spleen, yellowish liver discoloration, cardiomegaly (in PND 21 pups only at necropsy; not observed in PND4 pups macroscopically) **150 ppm (F0 M: 14 mg/kg bw; F premating: 16, gestation: 14; lactation 22 mg/kg bw; F1 M: 18mg/kg bw; F premating: 19, gestation: 14; lactation 22 mg/kg bw): F0 and F1:** <u>parental toxicity:</u> no treatment-related effect <u>developmental toxicity:</u> impaired body weight and body weight gain, decreased weight of thymus, yellowish liver discoloration, cardiomegaly (in	Dammann M (2015)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		PND 21 pups only at necropsy) **50 ppm (F0 M: 5 mg/kg bw; F premating: 5, gestation: 5; lactation 8 mg/kg bw; F1 M: 6 mg/kg bw; F premating: 6, gestation: 5; lactation 7 mg/kg bw): F0 and F1:** parental toxicity: no treatment-related effect developmental toxicity: no treatment-related effect **NOAEL (Systemic toxicity (parents):** 150 ppm (17 mg/kg bw/day) **NOAEL (Developmental toxicity):** 50 ppm (5 mg/kg bw/day in adults*; however, estimated actual doses in pups are 12 mg/kg bw/day due to simultaneous self-feeding*) **BMD calculations for body weight effects in F1 adult females and F2 pups:** $BMDL_{05}$ (females, PND 21): 25.5-45.3 mg/kg bw, (using measured substance intakes during lactation); $BMDL_{05}$ (pups, PND 21): 39.8 mg/kg bw (using estimated substance intakes for PND 21)	
Mechanistic study (no guideline available) Deviations: not applicable GLP: yes Species/strain: Wistar rat Sex: male Age: young (3 weeks) and adult (10 weeks) No. animals/dose: 10 males Effect on serum iron after 7 days of dietary intake	Test substance: Dimoxystrobin, purity: 98.4% (equivalent to substance in Chapter 1.1) Dose/concentrations: young rats: 0, 250, 500 ppm old rats: 0, 500 ppm Route of administration: oral, diet Duration of treatment: 7 days Control: yes, plain diet	**500 ppm:** adult rat (33.4 mg/kg bw/day): decreased food consumption, reduced serum iron level, thickening of the duodenum in 7/10 animals young rat (65.3 mg/kg bw/day): decreased food consumption, reduced serum iron level **250 ppm:** young rat (33.8 mg/kg bw/day): decreased food consumption and body weight, reduced serum iron level **NOAEL (adult rat, serum iron level):** < 500 ppm (33.4 mg/kg bw/day) **NOAEL (young rat, serum iron level):** < 250 ppm (33.8 mg/kg bw/day)	Anonymous, 2005a 2005/1004845
Enhanced one-generation repro-ductive toxicity study OECD 416 (2001); 2008/440/EC, Part B, L 142; EPA 870.3800 Deviations: study	Test substance: Dimoxystrobin, purity: 98.5% (equivalent to substance in Chapter 1.1) Dose/concentration: 0,	**50 ppm (4.3 mg/kg bw/day):** parental toxicity: no treatment-related effect **20 ppm (1.7 mg/kg bw/day):** parental toxicity: no treatment-related effect **10 ppm (0.9 mg/kg bw/day):**	Anonymous, 2011 2011/1211676

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
design was limited to 1 generation; estrus cycle and sperm parameters were not determined; organ weight determination, gross necropsy and histopathology were not included; haematology and determination of iron and transferrin was included for blood samples of parental animals taken before sacrifice as well as of pups on PND 7, 14, 21 GLP: yes Species/strain: Wistar rats Sex: male/female No. animals/sex/ dose: 25	10, 20, 50, ppm Route of administration: oral in feed Duration of treatment: ♂: 73 days prior mating, up to 2 weeks mating period ♀: 73 days prior mating, up to 2 weeks mating period, continuously exposed during gestation up to weaning (LD 21), however, during lactation exposure levels were reduced by 50% due to increased food consumption during that phase Control: yes, plain diet	<u>parental toxicity</u>: no treatment-related effect **NOAEL (Systemic toxicity (parents):** **50 ppm (4.3 mg/kg bw/day)**	
Mechanistic study (no guideline available) Deviations: not applicable GLP: no (screening study) Species/strain: Wistar rat Sex: male Age: adult (10 weeks) No. animals/dose: 6 males Effect on serum iron after 2 and 6 days of dietary intake	Test substance: Dimoxystrobin, purity: 98.4% (equivalent to substance in Chapter 1.1) Dose/concentrations: 0, 10, 50, 250, 500 ppm Route of administration: oral, diet Duration of treatment: 7 days Control: yes, plain diet	**500 ppm (~40 mg/kg bw/day):** reduced serum iron level **250 ppm (~20 mg/kg bw/day):** reduced serum iron level **50 ppm (~4 mg/kg bw/day):** no treatment-related effect on serum iron level **10 ppm (~1 mg/kg bw/day):** no treatment-related effect on serum iron level **<u>NOAEL (adult rat, serum iron level):</u>** 50 ppm (~4 mg/kg bw/day)	Anonymous, 2002a 2002/1014245 and 2003/1009198
Mechanistic study (no guideline available) Deviations: not applicable GLP: yes	Test substance: Dimoxystrobin, purity: 99.7% (equivalent to substance in Chapter 1.1) Dose/concentrations:	**22 ppm (3.42 mg/kg bw/day):** no treatment-related effect **11 ppm (1.71 mg/kg bw/day):** no treatment-related effect	Anonymous, 2010 2010/1026748

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Species/strain: Wistar rat Sex: male Age: young (3 weeks) No. animals/dose: 10 males Effect on serum iron and transferrin after 7 days of dietary intake	0, 6, 11, 22 ppm Route of administration: oral, diet Duration of treatment: 7 days Control: yes, plain diet	**6 ppm (0.95 mg/kg bw/day):** no treatment-related effect **NOAEL (young rat, serum iron level):** ≥ 22 ppm (3.42 mg/kg bw/day) **NOAEL (young rat, serum transferrin level):** ≥ 22 ppm (3.42 mg/kg bw/day)	

Table 28: Summary table of other studies relevant for STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Mechanistic study (no guideline available) Deviations: not applicable GLP: no (appropriate data documentation, not QA checked) Species/strain: Wistar rat Sex: male Age: adult (10 weeks) No. animals/dose: 10 males in the control group, 5 males in the treatment and recovery groups Effect on serum iron	Test substance: Dimoxystrobin, purity: 98.4% (equivalent to substance in Chapter 1.1) Dose/concentrations: 0, 4500 ppm Route of administration: oral, diet Duration of treatment: 3 or 5 weeks Frequency of treatment: 3 weeks of treatment followed by a 2 week recovery period Control: yes, plain diet	**4500 ppm:** treatment group (5 weeks: 264 mg/kg bw/day): reduced food consumption, body weight and body weight gain, evidence of hypochromic microcytic anaemia, reduced serum iron level, depletion of iron reserves recovery group (3 weeks: 232 mg/kg bw/day): The effect on serum iron levels was fully reversible. There was some but not complete recovery in haematological effects. **NOAEL (adult rat, serum iron level):** not applicable	Anonymous 2002a 2002/1005354

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

In the modified one-generation reproduction toxicity study after 4 weeks of test substance administration (before mating period) decreased haemoglobin, mean corpuscular volume (MCV), mean corpuscular

haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were found in the peripheral blood of the high dose males (1200 ppm). Increased red blood cell counts, anisocytosis, microcytosis and hypochromasia were also detected in these males. Moreover, in the mid dose males (500 ppm) MCV and MCH were reduced and microcytosis was increased. In the females, decreased MCH and MCHC values and slightly increased microcytosis and hypochromasia were found in the high dose animals at this time interval (see Table 50).

After 3 months of test substance administration (shortly before sacrifice) slightly increased microcytosis was seen in the high dose males (1200 ppm), only. In the peripheral blood of the high dose dams, decreased haemoglobin, MCV, MCH and MCHC values and increased red blood cells, platelets, reticulocytes, microcytosis and hypochromasia were found. In the dams of the mid dose group (500 ppm) decreases in MCH and MCHC as well as increases in microcytosis were detected. Slight effects were detected in the low dose group of 150 ppm.

On day 21 after birth the following haematology changes were observed in the high dose male and female F1 pups (1200 ppm): decreases in red blood cells, haemoglobin, haematocrit, MCV and MCH; increases in platelets, reticulocytes, microcytosis and anisochromasia. Moreover, in the high dose male pups MCHC was increased. In the male and female pups of the mid dose group (500 ppm) haemoglobin, haematocrit, MCV and MCH were decreased and reticulocytes, microcytosis, anisochromasia and normoblasts were increased. Significantly reduced erythrocytes were also measured in the blood of the mid dose male pups. In the low dose male pups (150 ppm) MCV was reduced and microcytosis and anisochromasia were increased. In the low dose female pups reticulocytes and microcytosis were elevated.

An overview on the haematological changes for parental animals and PND21 pups can be found in Table 51.

Table 29: Summary of haematology parameters in the modified one-generation study

Dose [ppm]	**Haematological parameters**						
Males, day 29	**RBC (TERA/L)**	**HGB (MMOL/L)**	**HCT (L/L)**	**MCV (FL)**	**MCH (FMOL)**	**MCHC (MMOL/L)**	**PLT (GIGA/L)**
0	7.71	9.3	0.411	53.3	1.21	22.69	819
150	7.49	9.1	0.399	53.4	1.22	22.79	800
500	7.87	9.1	0.404	51.4**	1.16**	22.47	856
1200	8.2**	8.5***	0.387	47.2***	1.04***	22.04**	896
Males, day 98							
0	8.84	9.5	0.484	54.7	1.08	19.68	709
150	8.6	9.5	0.474	55.2	1.11	20.09**	700
500	9	9.6	0.489	54.3	1.06	19.55	706
1200	8.97	9.4	0.479	53.4	1.05	19.63	723
Females, day 29							
0	7.68	9.4	0.409	53.3	1.22	22.94	740
150	7.58	9.3	0.403	53.1	1.22	22.59	768
500	7.72	9.2	0.403	52.2	1.19	22.78	728
1200	7.96	9	0.405	51	1.14**	22.28**	841
Females, day 100							
0	8.5	10.4	0.498	58.8	1.22	20.76	779
150	8.31	9.5**	0.471	56.7	1.14*	20.07*	763
500	9.09	10	0.505	55.7	1.11*	19.90***	793
1200	9.54*	9.4***	0.491	52.0*	1.00**	19.21***	981**
Male pups, PND 21							
0	4.64	5.4	0.305	65.9	1.17	17.67	825
150	4.67	5.1	0.286	61.4**	1.08	17.62	821
500	4.28*	3.9***	0.223***	52.0***	0.91***	17.48	1065
1200	3.10***	3.0***	0.152***	47.1***	0.97**	20.59*	1227**
Female pups, PND 21							
0	4.47	5.1	0.281	63	1.14	18.08	759
150	4.78	5.1	0.291	61	1.07	17.6	690
500	4.37	4.0***	0.230**	52.3***	0.91***	17.39	1084
1200	3.02**	2.7***	0.139**	44.5***	0.91***	20.65	1471*

*$p \leq 0.05$; ** $p \leq 0.02$; *** $p \leq 0.002$

Table 30: Overview of haematological changes in the modified one-generation study

Dose [ppm]	**150**	**500**	**1200**
Adult males	No effect	**Premating:** ↓ MCH (-4%), MCV (-4%); ↑ microcytosis	**Premating:** ↓ Hgb (-9%), MCV (-11%), MCH (-14%), MCHC (-3%); ↑ RBC (+6%), anisocytosis, microcytosis and hypochromasia, reticulocutes (+52%) **Study day 100:** slight↑ microcytosis
Adult females	**Study day 100:** ↓ Hgb (-9%), MCH (-7%), MCHC (-3%); ↑ microcytosis	**Study day 100:** ↓ MCH (-9%), MCHC (-4%); ↑ microcytosis, reticulocytes (+44%)	**Premating:** ↓ MCV (-3%), MCH (-7%), ↑ RBC (+4%), microcytosis, hypochromasia, reticulocytes (+20%) **Study day 100:** ↓ Hgb (-10%), MCV (-12%), MCH (-18%), MCHC(-7%); ↑ RBC (+12%), microcytosis, hypochromasia, platelets and reticulocytes (+333%)
Pups PND 21	↓MCV (M:-7%) ↑microcytosis and reticulocytes (M:+6%; F:+56%); ↑anisochromasia (males)	↓ Hgb (M:-28%; F:-22%), Hct (M:-27%; F:-18%), MCV (M:-21%; F:-17%), MCH (M:-22%,F: -20%); ↓ RBC (M:-8%); ↑ anoisochromasia, microcytosis, reticulocytes (M:+57%; F:+58%), normoblasts (both sexes)	↓ RBC (M: -33%; F:-32%), Hgb (M:-45%; F:-47%), Hct (M:-50%; F:-51%), MCV (M:-29%; F:-29%), MCH (M:-17%; F:-20%), MCHC (M: -19%); ↑ anisochromasia, microcytosis, platelets and reticulocytes (M:+66%; F:+216%)

The changes in haematological parameters observed are indicative for an iron-deficiency microcytic hypochromic anaemia. Dimoxystrobin reduces iron uptake in the duodenum and thus causes lower serum iron levels in rats and a microcytic hypochromic anaemia, which is characterized by reduced blood haemoglobin (HGB), reduced mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV). The correlation of anaemia and reduced iron levels was clearly shown in a mechanistic study, where a reduction in serum iron levels was accompanied by changes in haematological parameters indicative for anaemia (see Anonymous 2005a, 2005/1004845; Anonymous 2002a, 2002/1005354).

Anaemia was seen at the same dietary concentrations in pups and parental animals. However, the individual haematology parameters were more pronounced in the PND 21 pups.

This is not considered to be indicative for a higher susceptibility in the pups, because

- the milk of anaemic dams contains less iron compared to control dams,
- pups are lacking body iron stores, and
- pups were exposed to higher dimoxystrobin doses (see above; estimated pup intake is 260 mg/kg bw, while parental females consume 170.5 mg/kg bw during lactation; see Table 52).

Table 31: Approximate mg/kg bw/day compound exposure to parental animals and estimated mg/kg bw/day exposure to pups during last week of lactation (excluding amount, if any, transferred in milk) in the modified one-generation study

ppm in diet	**0**	**150**	**500**	**1200**
Male (pre-mating)	0	17.5	56.6	128.4
Female (pre-mating)	0	18.3	58.2	131.9
Female (gestation)	0	14.5	47.4	109.3
Female (lactation)[a)]	0	23.3	82.8	170.5
Pups [b)]	0	36	114	260
Pups (not corrected)[c)]	0	18	57	130

Milk is generally a poor source of iron, milk of dams suffering from iron deficiency anaemia contains less iron than usual (e.g. 34 µg/g dry wt in controls vs. 22 µg/g dry wt in treated dams; Roth and Smith, 1988), so that these dams are even less able to transfer sufficient iron to the young via the milk in the early postpartum period (Anaokar and Garry, 1981).

Compared to adult animals, that can store excessive amounts of iron in tissues in two forms, ferritin or hemosiderin, which can be mobilized in an iron deficient state, pups have physiologically only very small iron stores. Therefore, the body iron stores of nursing pups can easily be depleted since their blood volume expands to accommodate the increasing body size (Roth and Smith, 1988).

The iron-deficiency anaemia is occurring in dams and offspring of the reproduction toxicity studies at the same dose levels (≥ 150 ppm). The susceptibility of the pups to develop anaemia is not higher compared to the adults, as the iron deficiency is the first event to occur after dimoxystrobin treatment and calculations show, that the maternal and offspring BMDL values are comparable.

The slight effects on haematological parameters observed in the 150 ppm parental animals indicate a beginning anaemia, which is treatment-related, but not considered to represent an adverse outcome, thus the lowest NOAEL for parental toxicity is considered to be 150 ppm (about 18 mg/kg bw/day).

The correlation of anaemia and reduced iron levels was clearly shown in a rat 5-week feeding study dosed with 4500 ppm dimoxystrobin (Anonymous 2002a, 2002/1005354), where severe anaemia was preceded by decreased serum iron levels detected already 24 hours after start of treatment. After cessation of treatment iron levels increased above control levels and a considerable, however not complete recovery of anaemia was noted (see Tables below).

Table 32: Serum iron levels in a 5-week feeding study in rats (4500 ppm)

		Serum iron concentration (µmol/L)								
Dose [ppm]		**5 d before admin**	**Admin for 1 d**	**Admin for 2 d**	**Admin for 5 d**	**Admin for 15 d**	**Admin for 30 d**	**admin for 36 d**	**Admin for 19 d + 11 d recovery**	**Admin for 19 d + 17 d recovery**
Control	Mean	42.2	52.8	46.8	57.3	45.9	37.2	43.0	37.2	43.0
4500 232 mg/kg bw/day	Mean	48.5	**25.6****	**25.4****	**14.7****	**16.2****	**24.4***	**13.4****	55.9	54.9
	% dev.	+15	-52	-46	-74	-65	-34	-69	+50	+28

*p ≤ 0.05; ** p ≤ 0.01

Table 33: Hematological parameters in a 5-week feeding study in rats (4500 ppm)

		Hematological examinations			
Dose [ppm]		**HGB (mmol/L)**	**MCV (fl)**	**MCH (fmol)**	**MCHC (mmol/L)**
Control (30 d)	Mean	9.4	56.3	1.17	20.79
4500 (30 d)	Mean	**7.6****	**44.3****	**0.89****	**20.02****
	% dev.	-19.1	-21.3	-23.9	-3.7
4500 (19 d + 11 d recovery)	Mean	**8.7***	**48.7****	**1.01****	20.67
	% dev	-7.4	-13.5	-13.7	-0.6

*p ≤ 0.05; ** p ≤ 0.01

In the modified one generation study the mean terminal body weight was significantly decreased in males of mid and high dose groups showing dose response relationship. This was regarded as treatment-related. In females of the high dose group, the mean terminal body weight was slightly decreased (-4.7%), however, this was not significant. In contrast, in females of the low dose group, the mean terminal body weight was slightly although significantly increased. This was, however, regarded as unrelated to treatment.

In males of the mid and high dose groups, the mean weight of heart was slightly although significantly decreased. This was regarded to be related to the decreased mean terminal body weight (see Table 55).

Table 34: Absolute and relative organ weights of F1 males (Mean ± SD#)

Dose group		0	150	500	1200
Terminal bw [g]	absolute	366 ± 27	362 ± 30	**325 ± 26****	299 ± 19
	relative	-	-	-	-
Liver [g]	absolute	9.03 ± 1.02	8.63 ± 0.85	8.25 ± 0.73	8.45 ± 0.39
	relative	2.47 ± 0.18	2.38 ± 0.10	2.54 ± 0.11	**2.83 ± 0.18****
Heart [g]	absolute	1.07 ± 0.10	1.04 ± 0.08	**0.96 ± 0.08***	**0.96 ± 0.08***
	relative	0.292 ± 0.017	0.288 ± 0.019	0.295 ± 0.010	**0.323 ± 0.028****

#: numbers were rounded and thus may not exactly reflect the numbers given in the study report
** p ≤0.05, ** p ≤0.01 (Kruskal-Wallis and Wilcoxon-test (two-sided))*

In females of the high dose group, the mean liver weight was slightly although significantly increased. In females of the low dose group, the mean weights of liver and spleen were significantly increased. This was regarded to be related to the increased mean terminal body weight rather than to treatment (see Table 56).

Table 35: Absolute and relative organ weights of F1 females (Mean ± SD#)

Dose group		0	150	500	1200
Terminal bw [g]	absolute	202.8 ± 17.4	224.5 ± 21.0	205.0 ± 17.4	193.2 ± 13.4
	relative	-	-	-	-
Liver [g]	absolute	5.98 ± 1.36	**6.98 ± 0.74***	6.76 ± 1.48	**7.16 ± 1.30***
	relative	2.94 ± 0.56	3.13 ± 0.40	3.29 ± 0.59	**3.69 ± 0.49****
Heart [g]	absolute	0.816 ± 0.120	0.868 ± 0.066	0.874 ± 0.094	0.872 ± 0.043
	relative	0.402 ± 0.043	0.389 ± 0.037	0.427 ± 0.042	**0.452 ± 0.022***

#: numbers were rounded and thus may not exactly reflect the numbers given in the study report
** $p \leq 0.05$, ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test (two-sided))*

The other mean absolute weight parameters did not show significant differences when compared with the control group.

Due to the significantly decreased mean terminal body weight, the mean relative weights of liver and heart (males, high dose group) were significantly increased. This was not considered treatment-related.

In females of the high dose group, the mean relative weights of liver and heart were also significantly increased. Although the decrease of the mean terminal body weight in this group was not significant, the increased mean weights of both organs are interpreted to be related to the decreased mean terminal body weight rather than to a treatment-related effect. The other mean relative weight parameters did not show significant differences when compared with the control group.

A comparison of the cardiac effects in parental animals and pups can be found in Table 57.

Table 36: Overview on cardiac effects in the modified one-generation study

Dose [ppm]	**150**	**500**	**1200**
Parental	No effect	Absolute heart weight slightly decreased (m);	Absolute heart weight slightly decreased (m) (related to decreased bw); ↑Relative heart weights due to decreased bw (not treatment-related) Gross lesions: no effects on hearts
Pups PND 21	No effect	↑Relative heart weight (20%) (males statistically significantly increased)	↑Relative heart weight (57%) (males and females statistically significantly increased)
Pups Necropsy findings PND21	No effect	-	Cardiomegaly (only in PND21 pups, not PND4)

Adaptive thickening of the wall of the duodenum was noted in one male of the high dose group, and also in a mechanistic study (Anonymous 2005a, 2005/1004845) in 7 of 10 adult animals at 500 ppm this modification

was observed. This observation was regarded to be treatment-related. A few other gross lesions were noted in the glandular stomach (erosion ulcer in each one female of all groups), mammary gland (mass in a low dose female), and axillary and iliac lymph nodes (enlarged in a low dose group female). They were all regarded incidental and unrelated to treatment.

In the two generation toxicity study alterations of absolute and/or relative organ weights in F0 and F1 parental rats were attributed to decreased body weights (see Chapter 10.10.2.). None of the organs with weight changes in the adults showed correlating histopathology. An overview over the most obvious necropsy observations in F1 and F2 pups, considered to be treatment-related, is given in Table 21 (see Chapter 10.10.5.).

The impaired body weight gain and the necropsy observations in offspring, as discussed in the Chapter 10.10. Reproductive toxicity, are the consequence of an iron-deficient anaemia, occurring after direct (via feed or milk) exposure with dimoxystrobin.

10.12.2 Comparison with the CLP criteria

STOT-RE is assigned on the basis of a substance demonstrating evidence of significant or severe toxicity, generally at or below the oral guidance value of 100 mg/kg/d (for a classification in category 2) obtained in a 90-day rat study. The oral guidance value for a classification in category 1 is ≤ 10 mg/kg/d. 'Significant' toxicity is taken to mean changes that clearly indicate functional disturbance or morphological changes that are toxicologically relevant. 'Severe' toxicity is considered to be more profound or serious and indicates changes that are of a considerably adverse nature with a significant impact on health.

In accordance with the guidance on the application of the CLP criteria, the following effects might be indicative of significant or severe toxicity and thus merit classification for STOT-RE.

a) Morbidity or death resulting from repeated or long-term exposure.
b) Significant functional changes in the central or peripheral nervous systems or other organ systems
c) Any consistent and significant adverse change in clinical biochemistry, haematology or urinalysis parameters
d) Significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination
e) Multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity
f) Morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in liver)
g) Evidence of appreciable cell death in vital organs incapable of regeneration

In the relevant studies changes in bodyweight gain and food consumption with toxicological importance was observed but do not, by themselves, indicate "significant" toxicity. Also changes in organ weights occurred with no evidence of organ dysfunction.

In the modified one-generation reproduction toxicity study after 100 days of test substance administration in the dams of the mid dose group (500 ppm) decreases in MCH and MCHC as well as increases in microcytosis were detected. **The effective dose identified at 500 ppm (57 mg/kg bw/day).** The effective doses extrapolated for 90 days are shown below in Table 58.

Table 37: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Anonymous, 2001,	57	100 days (females)	63	STOT-RE

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
2000/1016870				Category 2

The correlation of anaemia and reduced iron levels was clearly shown (Anonymous 2002a 2002/1005354). After cessation of treatment iron levels increased above control levels and a considerable, however not complete recovery of anaemia was noted.
A treatment related duodenum wall thickening considered as an adaptive change therefore does not fulfil criterion f).
Thus, the criterion c) of the above bulleted criteria would apply for the available data set of dimoxystrobin as the extrapolated effective dose for adverse hematological effects for females are 63 mg/kg bw/day which support STOT-RE Category 2 classification according the guidance (oral route, rat, 10≤ effective dose mg/kg bw/d ≤ 100). Furthermore, as the anaemia not completely irreversible and the question whether dimoxystrobin simply affected the absorption rate of iron and/or whether some other mechanism was involved e.g. possible chelation of iron by dimoxystrobin/metabolites in food and/or serum is not clarified, thus STOT-RE Category 2, H373 harmonised classification proposed.

10.12.3 Conclusion on classification and labelling for specific target organ toxicity – repeated exposure

Classification proposed as STOT-RE 2; H373 (May cause damage to organs (blood) through prolonged or repeated exposure).

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

DS proposed classification of dimoxystrobin as STOT RE 2; H373 (may cause damage to blood through prolonged or repeated exposure) based on reduction of iron levels and partially irreversible anaemia. In the modified one-generation reproduction toxicity study after 100 days of test substance administration in the dams of the mid dose group (57 mg/kg bw/day) decreases in mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) as well as increases in microcytosis were detected. The effective doses extrapolated for 90 days corresponded to 63 mg/kg bw/day.

Comments received during consultation

One MSCA highlighted that the comparison with guidance values was focused on doses that did not clearly evoke significant/severe effects and recommended to consider not only the doses of the modified one-generation reproduction toxicity study, but also those of other studies (e.g. short-term toxicity studies in rats, mice and dogs). DS replied that the results of the 90-day studies in rat, mouse and dog and 1-year study in dogs showed no effects or

minor effects that were not sufficiently severe to warrant a classification for STOT RE.

Assessment and comparison with the classification criteria

Modified one-generation reproductive toxicity study (Anonymous, 2001, 2000/1016870)

A modified one-generation reproductive toxicity study was performed according to OECD TG 415 (1983) and GLP. Ten males and ten females Wistar rats were dosed with dimoxystrobin (purity 98.4%) at 1200, 500 and 150 ppm (equivalent to 130, 57 and 18 mg/kg bw/day; respectively). Males were treated during the 47-day premating period and 2-week mating period. Females were treated during the 47-day premating period, 2-week mating period and gestation period up to lactation day 21. The table below shows an overview on the haematological changes in parental animals and pups at PND 21.

After 4 weeks of test substance administration (before the mating period) decreased haemoglobin (HGB), mean corpuscular volume (MCV), MCH and mean corpuscular haemoglobin concentration (MCHC) were found in the peripheral blood of the high dose males (1200 ppm). Increased red blood cell counts (RBC), anisocytosis, microcytosis and hypochromasia were also detected in these males. Moreover, in the mid dose males (500 ppm) MCV and MCH were reduced and microcytosis was increased. In the females, decreased MCH and MCHC values and slightly increased microcytosis and hypochromasia were found in the high dose animals at this time interval (see table below). After 3 months of test substance administration (shortly before sacrifice), slightly increased microcytosis was seen in the high dose males (1200 ppm), only. In the peripheral blood of the high dose dams, decreased HGB, MCV, MCH and MCHC values and increased RBC, platelets (PNT), reticulocytes, microcytosis and hypochromasia were found. In the dams of the mid dose, group (500 ppm) decreases in MCH and MCHC as well as increases in microcytosis were detected. Slight effects were detected in the low dose group of 150 ppm. On day 21 after birth the following haematology changes were observed in the high dose male and female F1 pups (1200 ppm): decreases in RBC, HGB, haematocrit (HCT), MCV and MCH; increases in PNT, reticulocytes, microcytosis and anisochromasia. Moreover, in the high dose male pups MCHC was increased. In the male and female pups of the mid dose group (500 ppm) HGB, HCT, MCV and MCH were decreased and reticulocytes, microcytosis, anisochromasia and normoblasts were increased. Significantly reduced erythrocytes were also measured in the blood of the mid dose male pups. In the low dose male pups (150 ppm) MCV was reduced and microcytosis and anisochromasia were increased. In the low dose female pups, reticulocytes and microcytosis were elevated.

***Table**: Summary of haematology parameters in the modified one-generation study*. RBC = Red Blood Cells; HGB = Haemoglobin; HCT = Haematocrit; MCV = Mean Corpuscular Volume; MCH = Mean corpuscular haemoglobin; MCHC = Mean corpuscular haemoglobin concentration; PNT = Platelet *p ≤ 0.05; ** p ≤ 0.02; *** p ≤ 0.002

Dose [ppm]	RBC (TERA/L)	HGB (MMOL/L)	HCT (L/L)	MCV (FL)	MCH (FMOL)	MCHC (MMOL/L)	PLT (GIGA/L)
Males, day 29							
0	7.71	9.3	0.411	53.3	1.21	22.69	819
150	7.49	9.1	0.399	53.4	1.22	22.79	800
500	7.87	9.1	0.404	51.4**	1.16**	22.47	856
1200	8.2**	8.5***	0.387	47.2***	1.04***	22.04**	896

	(↑6%)	(↓9%)		(↓12%)	(↓14%)	(↓3%)	
Males, day 98							
0	8.84	9.5	0.484	54.7	1.08	19.68	709
150	8.6	9.5	0.474	55.2	1.11	20.09**	700
500	9.0	9.6	0.489	54.3	1.06	19.55	706
1200	8.97	9.4	0.479	53.4	1.05	19.63	723
Females, day 29							
0	7.68	9.4	0.409	53.3	1.22	22.94	740
150	7.58	9.3	0.403	53.1	1.22	22.59	768
500	7.72	9.2	0.403	52.2	1.19	22.78	728
1200	7.96	9.0	0.405	51.0	1.14** (↓7%)	22.28** (↓3%)	841
Females, day 100							
0	8.5	10.4	0.498	58.8	1.22	20.76	779
150	8.31	9.5**	0.471	56.7	1.14*	20.07*	763
500	9.09	10.0	0.505	55.7	1.11*	19.90***	793
1200	9.54* (↑12%)	9.4*** (↓10%)	0.491	52.0* (↓12%)	1.00** (↓18%)	19.21*** (↓8%)	981** (↑26%)
Male pups, PND 21							
0							
150	4.64	5.4	0.305	65.9	1.17	17.67	825
500	4.67	5.1	0.286	61.4**	1.08	17.62	821
1200	4.28* 3.10*** (↓33%)	3.9*** 3.0*** (↓44%)	0.223*** 0.152*** (↓50%)	52.0*** 47.1*** (↓29%)	0.91*** 0.97** (↓17%)	17.48 20.59* (↑16%)	1065 1227** (↑49%)
Female pups, PND 21							
0	4.47	5.1	0.281	63	1.14	18.08	759
150	4.78	5.1	0.291	61	1.07	17.6	690
500	4.37	4.0***	0.230***	52.3***	0.91***	17.39	1084
1200	3.02** (↓35%)	2.7*** (↓47%)	0.139*** (↓51%)	44.5*** (↓29%)	0.91*** (↓20%)	20.65	1471* (↑94%)

Mechanistic study: Effect of dimoxystrobin on serum iron (2002a, 2002/1005354)

Dimoxystrobin (98.4% purity) was administered in the diet to 10-week old male Wistar rats at 0 (10 rats) and 4500 ppm (equivalent to 232 mg/kg bw/day) (5 rats) for 3 weeks and was followed by a 2-week recovery period. The effect of the treatment on serum iron levels and on haematological parameters is shown in the tables below. Severe reductions in iron concentration were detected as early as 24 hours after beginning of the dimoxystrobin administration and this reduction was observed until day 36. The iron levels returned to normal and even higher levels than in control during the recovery period; however, no complete recovering of anaemia was noted during the recovery period.

Table: *Serum iron levels in a 5-week feeding study in rats (4500 ppm)* shown as mean of serum iron concentrations (µmol/l). The dose (4500 ppm) was equivalent to 264 mg/kg bw/day. D = day; D -5 = 5 days before administration; * = p<0.05; ** = p<0.01.

Dose [ppm]	D -5	D1	D2	D5	D15	D30	D36	D19 + recovery 11 D	D19 + recovery 17 D
Control	42.2	52.8	46.8	57.3	45.9	37.2	43.0	37.2	43.0
4500 ppm	48.5	**25.6**** ↓52%	**25.4**** ↓46%	**14.7**** ↓74%	**16.2**** ↓65%	**24.4*** ↓34%	**13.4**** ↓69%	55.9	54.9

Table: *Haematological parameters in a 5-week feeding study in rats (4500 ppm)* shown as means of respective parameters. The dose (4500 ppm) was equivalent to 264 mg/kg bw/day in the animals exposed for 30 days and 232 mg/kg bw/day in animals exposed for 19 days.
* = p<0.05; ** = p<0.01.

Dose [ppm]	HGB (mmol/l)	MCV (fl)	MCH (fmol)	MCHC (mmol/L)
Control (day 30)	9.4	56.3	1.17	20.79
4500 ppm (day 30)	**7.6**** ↓19%	**44.3**** ↓21%	**0.89**** ↓24%	**20.02**** ↓4%
4500 ppm (day 19 + 11 days recovery)	**8.7*** ↓7.4%	**48.7**** ↓13.5%	**1.01**** ↓13.7%	20.67

Mechanistic study: Determination of serum iron concentration in young and adult male Wistar rats after 7-day oral administration in the diet (2005a, 2005/1004845)

Dimoxystrobin (98.4% purity) was administered to groups of 10 young (3 weeks old) and adult (10 weeks old) male Wistar rats at dietary concentrations of 0 and 500 ppm over a period of 7 days. An additional group of young animals treated with 250 ppm was added. The dose of 500 ppm was equivalent to 65.3 and 33.8 mg dimoxystrobin/kg bw/day in young and adult animals, respectively. The dose of 250 ppm in young animals was equivalent to 33.8 mg/kg bw/day.

All animals showed significant reduction of iron concentration in serum after 2 and 7 days of dimoxystrobin treatment compared to control animals (table below). A thickening of the duodenum was observed during macroscopic examination in seven rats from group 3 (10-week old animals receiving 500 ppm of dimoxystrobin). This finding is considered as being related to treatment. No duodenum findings were observed in the other animal groups.

Table: *Serum iron concentration (µmol/l) of male rats (3 and 10 weeks old) after administration of dimoxystrobin for 2 and 7 days.* ** = p<0.01

Animals	Day	Dose (mg/kg bw/day) 0	33.8	65.3
3-weeks	2	92.6±7.5	56.0±27.8** (↓42%)	35.8±14.0** (↓61%)
	7	95.3±6.0	59.7±23.3** (↓38%)	33.6±13.1** (↓65%)
10-weeks	2	43.1±5.9	34.2±3.1** (↓21%)	
	7	44.0±3.5	36.0±3.5** (↓18%)	

Comparison with the criteria

The changes in haematological parameters observed (tables above) are indicative for an iron-deficiency microcytic hypochromic anaemia. Dimoxystrobin reduces iron levels in serum (tables above) and thus causes microcytic hypochromic anaemia, which is characterized by reduced HGB, MCH and MCV. The correlation of anaemia and reduced iron levels was clearly shown in a mechanistic study, where a reduction in serum iron levels was accompanied by changes in haematological parameters indicative for anaemia.

According to CLP criteria, any consistent and significant adverse change in clinical biochemistry or haematology shall be considered for classification. However, RAC notes that the severity of haematological effects in the parental animals in the modified one-generation reproductive toxicity study does not warrant a classification. The mechanistic study for testing the effects of dimoxystrobin on serum iron shows acute reductions in serum iron concentration that does not seem to progress over time. In this same study it was demonstrated that the large reduction in iron concentration was causing less than 20 % differences in HGB, MCV, MCH and MCHC values as compared to control animals. RAC concludes that these effects are not of sufficient severity to warrant classification. The second mechanistic study for the determination of serum iron concentration in adult male Wistar rats after 2 and 7-day oral administration in the diet did not show effects warranting classification.

The modified one-generation reproductive toxicity study suggests that pups might be more susceptible than adults to haematological alterations induced by dimoxystrobin. This higher sensitivity of young animals as compared to adults is also shown in the mechanistic study in which the magnitude of effects on serum iron concentrations is doubled in 3-week old rats as compared to 10-week old rats. RAC concludes that the effects in pups are relevant for the assessment of reproductive toxicity rather than for STOT RE.

RAC notes that DAR includes additional information on haematological effects of dimoxystrobin; this is considered further in the BD.

Overall, RAC concludes that the severity of anaemia observed in adult rats **does not warrant a STOT RE classification.**

Supplemental information - In depth analyses by RAC

RAC notes that DAR includes additional information on haematological effects of dimoxystrobin. The 3-month oral toxicity study in rats showed alterations in different haematological parameters ranging between and 2 and 6% at doses of 300 mg/kg bw/day. Effects of similar magnitude were reported in the 3-month oral toxicity study in mice but at doses of 2867 mg/kg bw/day. Moreover, the 4-week dermal toxicity study in rats showed no substance-related effects on haematology after exposure of 1000 mg/kg bw/day. Thus, RAC concludes that none of the effects reported in repeated dose toxicity studies in mouse and rat support a classification for STOT RE.

The 3-month oral toxicity study in dogs showed 2-7% variation of red blood cells at 36.8 mg/kg bw/day, and similar variations were reported in the 12-month oral toxicity study in dogs after exposure to 22.3 mg/kg bw/day. The effects in dogs were reported within the guidance range for classification. However, RAC concludes that the magnitude of the effects is too low for warranting classification for STOT RE.

10.13 Aspiration hazard

Not relevant as no changes to the existing harmonized classification for dimoxystrobin are proposed.

10.13.1Conclusion on classification and labelling for aspiration hazard

The substance is **not classified for aspiration hazard**.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 38: Summary of relevant information on rapid degradability

Method	Results				Reference
Ready biodegradability					
Manometric respirometry test OECD 301 F	Not readily biodegradable 0-10% degradation after 28 d. Study valid				Werner, 1999 1999/10288
Hydrolysis as a function of pH US-EPA, 161-1	Hydrolytically stable at all tested pH values (pH 4 - pH 9) and temperatures (25°C and 50°C). Study duration 5 d (50°C) or 30 d (25°C). Study valid				McKenna & Baucom, 1997 1996/5244
Aerobic mineralisation in surface water OECD 309	No significant degradation was observed in the pelagic test. 89-97% of a.i. still present after 59 d. < 1% mineralisation after 59 d. Study valid				Yeomans, 2014 2014/1031018
Water/sediment study: aerobic transformation in aquatic sediment systems SETAC (1995), BBA (Part IV,5-1) and EPA (Subdivision N, Series 162-4)	**Test System**	**DT_{50} water phase [d]**	**DT_{50} sediment [d]**	**DT_{50} total system [d]**	
	Dark conditions Berghäuser Altrhein Kellmetschweiher (20°C, 100 d) 0.8-2.1% minerali-sation after 100 d Study valid	 14 25	 n.c. n.c.	 298 835	Ebert, 2000 2000/1000121 Fent, 2001 2000/1014987 kinetic evaluation of the two water/sediment studies: Budde, 2015 2014/1133879
	Outdoor conditions (natural sunlight) Kellmetschweiher (18.1°C, mean, 120d) Study valid	19	101	27	
Aqueous photolysis US-EPA, 161-2	$T_{1/2}$ = 63 d (sterile buffer solution), pH 7, 22°C, 15 d) 0.1-1.2% mineralisation after 15 d $T_{1/2}$ = 14 d (natural surface water), pH 8.6, 22°C, 15 d) No information on mineralisation (Test item not radio-labelled) Study valid				Singh, 1998 1997/5286 Goetz & Moss, 1998 1997/5428 kinetic evaluation of the two photolysis studies: Budde, 2014 2014/1263218

11.1.1 Ready biodegradability

The aerobic biodegradability of dimoxystrobin was evaluated in the "Manometric Respirometry Test" according to OECD guideline 301/F (biochemical oxygen demand). Activated sewage sludge was used for the test incubation carried out with a dimoxystrobin concentration of 100 mg/l. Between 0 and 10% degradation was observed over the 28 day test duration. Study validity criteria were met.

Dimoxystrobin was found to be not readily biodegradable in this test.

11.1.2 Hydrolysis

The hydrolytic stability of dimoxystrobin was studied following US EPA Subdivision N Guideline, Series 161-1. Sterile aqueous buffer solutions (pH 4 5, 7 and 9) were prepared containing [14C-benzyl] dimoxystrobin (1.3 mg a.s./l). Samples (25 ml) of each treated buffer were then incubated at 25°C (pH 5, 7 and 9) or 50°C (pH4, 7 and 9) in flasks in the dark for 5 (incubation at 50°C) or 30 days (incubation at 25°C). At selected time intervals samples of each buffer were analysed directly by HPLC with the identity of parent dimoxystrobin confirmed by MS. Study validity criteria were met.

Dimoxystrobin proved to be hydrolytically stable at all tested pH values (pH 4 - pH 9) and temperatures (25°C and 50°C). Only trace amounts of unknown disintegration products (<1.6%) were detected. Hydrolysis is unlikely to be a major route of degradation at environmentally relevant temperature and pH.

11.1.3 Other convincing scientific evidence

11.1.3.1 Water, water-sediment and soil degradation data (including simulation studies)

Study 1: Aerobic mineralisation in surface water

The study was performed according to the OECD guideline 309 (Aerobic mineralization in surface water – Simulation biodegradation test). The pelagic test system was chosen for this study (surface water only). The test was performed at two different dimoxystrobin concentrations (10 µg/l and 90 µg/l), using two differently 14C-labelled test items (phenyl and benzyl label). The test vessels were attached to a flow-through system for continuous aeration and incubated at a temperature of 20°C in the dark. Samples were taken up to 59 days after treatment. The amount and nature of radioactivity in the water samples was determined by liquid scintillation counting (LSC) and chromatographic methods (radio-HPLC). Volatiles were trapped in 2 M sodium hydroxide and were also analysed by LSC. Parent substance and metabolite identification was done by co-chromatography with the corresponding reference items on HPLC. Study validity criteria were met.

In the aerobic aquatic mineralisation study (OECD 309), dimoxystrobin proved to be stable. After 59 days, 89 to 97% of the applied radioactivity was recovered as unchanged active substance. Only trace amounts of known metabolites 505M08 and 505M09 (< 1%) were detected. Other degradation products were detected only in minor amounts (< 1.5%). Mineralisation was negligible with < 1%. Dimoxystrobin was not significantly degraded in the natural water environment provided in the test.

Study 2: Degradation in aerobic aquatic environment

An aerobic water/sediment study was conducted according to SETAC (1995), BBA (Part IV,5-1) and EPA (Subdivision N, Series 162-4) guidelines. Two natural systems of water and sediment were used. The water/ sediment systems were taken from a pond (Kellmetschweiher, System A) and a pond-like side arm of a river (Berghäuser Altrhein, System B) respectively, both in Rhineland-Palatinate, Germany. [14C-Phenyl] and [14C-benzyl]dimoxystrobin were used and applied separately to the test systems. Test vessels contained 290ml (ca. 6cm depth) water and 190-200g wet weight (2-2.5cm depth) sediment. Dimoxystrobin was applied to the water at a rate of 0.103 mg a.s./l. The test vessels were incubated in the dark at a temperature of 20°C for up to 100 days. Aeration was achieved by a stream of CO_2 free air over the water surface. Radioactivity in the water was quantified directly by LSC and analysed by HPLC and HPTLC. Radioactivity in sediment extracts was quantified by LSC and analysis by HPLC and HPTLC. Radioactivity in the extracted sediment was quantified by combustion and LSC. Study validity criteria were met.

In a water/sediment study (OECD 308) under dark conditions, the behaviour of dimoxystrobin was characterized by a rather fast movement from the water to the sediment. No major metabolites were formed in the water or sediment phase of either system. The metabolites 505M08 and 505M09 were detected only in the water phase and only in very low amounts (≤ 5%, mean of two labels). In the sediment, no metabolites could be detected at any sampling time. Mineralization was low in both investigated systems (<2.5%) and no other volatile degradation products were detected. The bound residues in the sediment were formed only to a small extent, reaching 6.3 - 10.7%. The evaluation of the degradation rates of dimoxystrobin resulted in DT_{50}

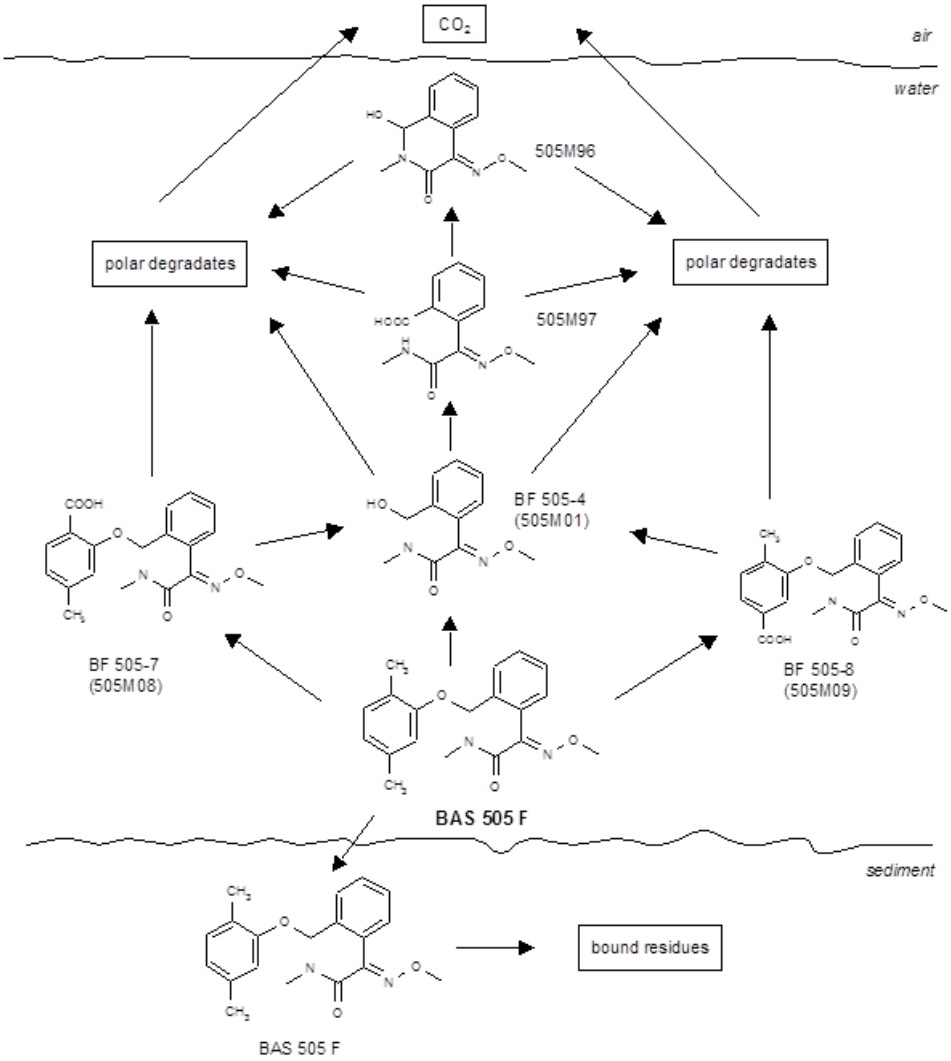
values for the water of 14 and 25 days (2 labels, 2 systems). The DT_{50} values for the total system were calculated to be 298 and 835 days.

Study 3: Degradation and distribution in a water-sediment system under outdoor conditions

An outdoor aerobic sediment/water study was conducted (natural light exposure) according to GLP. There are no agreed guidelines available for a study design of this type. The water/sediment system for this study was taken from Kellmetschweiher, the same site as for the water sediment studies conducted under dark conditions. Test vessels were filled with about 2.0 cm sediment (about 400 g wet weight) and a water layer of about 20 cm height (1950 ml). The system was allowed to equilibrate for 9 days before treatment. The water surface was treated with [14C-benzyl]dimoxystrobin at a rate of 140 µg per test vessel (ca. 71 µg/l). The water/sediment systems were placed in large isolated plastic tanks, filled with water in order to simulate a bigger water body with respect to temperature. The tanks were located in an ambient outdoor lysimeter facility in Neustadt an der Weinstrasse, Germany with outdoor temperature and light conditions (treatment date 5th July, 2000). In order to protect the vessels from rainfall they were placed under a special plexiglass cover which allowed UV and visible light transmission. If no rainfall was forecasted the plexiglass cover was removed. Samples were taken at selected time intervals up to 120 days after treatment. The water was analysed directly by HPLC. The sediment was extracted, and the extracts were analysed by HPLC. Volatiles could not be trapped. The mean water temperature during the experiment was 18.1°C (8.6-28.5°C). The measured average daily global radiation during the experiment was in the range 25-300 w/m^2. Study validity criteria were met.

In the outdoor water/sediment study, the degradation of dimoxystrobin in the total system was much faster (DT_{50} 27 days). Besides metabolites 505M08 (max. 3.6%) and 505M09 (max. 5.3%), also cleavage products 505M01 (max. 3.2%) and 505M96 (max. 9.6%) were detected in the water phase. In sediment, only trace amounts of metabolites were detected (≤ 1.2%). The non-extractable residues amounted up to 24%. Although volatile products were not collected, the decreasing material balance indicated mineralization of ~20% after 120 d.

Figure 1: Proposed route of degradation of dimoxystrobin in water and sediment under outdoor conditions


CO2
air
water
505M96
polar degradates
polar degradates
505M97
BF 505-4
(505M01)
BF 505-7
(505M08)
BF 505-8
(505M09)
BAS 505 F
sediment
bound residues
BAS 505 F

11.1.3.2 Photochemical degradation

Study 1: Photolysis in aqueous media

The aqueous photolysis of dimoxystrobin in a sterile buffer was studied according to US EPA Subdivision N Guideline, Series 161-2). The direct photolysis was performed with both [14C-benzyl] and [14C-phenyl]dimoxystrobin. The study was performed in a pH 7 buffer. The concentration of the active substance in the sterile aqueous buffer solution was 2.03 mg/l for the benzyl-label and 1.87 mg/l for the phenyl-label. For each label a separate experiment was performed. Sterilised glass vessels (20 ml volume) with quartz glass caps containing about 18 ml test solution were irradiated in a thermostated block. Each vessel had an air inlet and an air outlet. The incoming air was moistened, sterilised, and the CO2 was removed. For each label, four vessels were filled with treated buffer solution and placed in one row in the thermostated block. A trapping system for volatiles was connected to each row. The thermostated vessels were located under a xenon lamp with a light intensity of about 1664 µE/m2/sec and a cut-off for wavelengths < 290 nm to simulate natural sunlight. The duration of the experiment was 15 days with continuous irradiation. Appropriate volumes of each test solution were stored in a climatic chamber to be used as dark control. The temperature was 22°C during the experiments. Samples were analysed by HPLC with analyte identity confirmation by MS. Trapped volatiles were quantified by LSC. Study validity criteria were met.

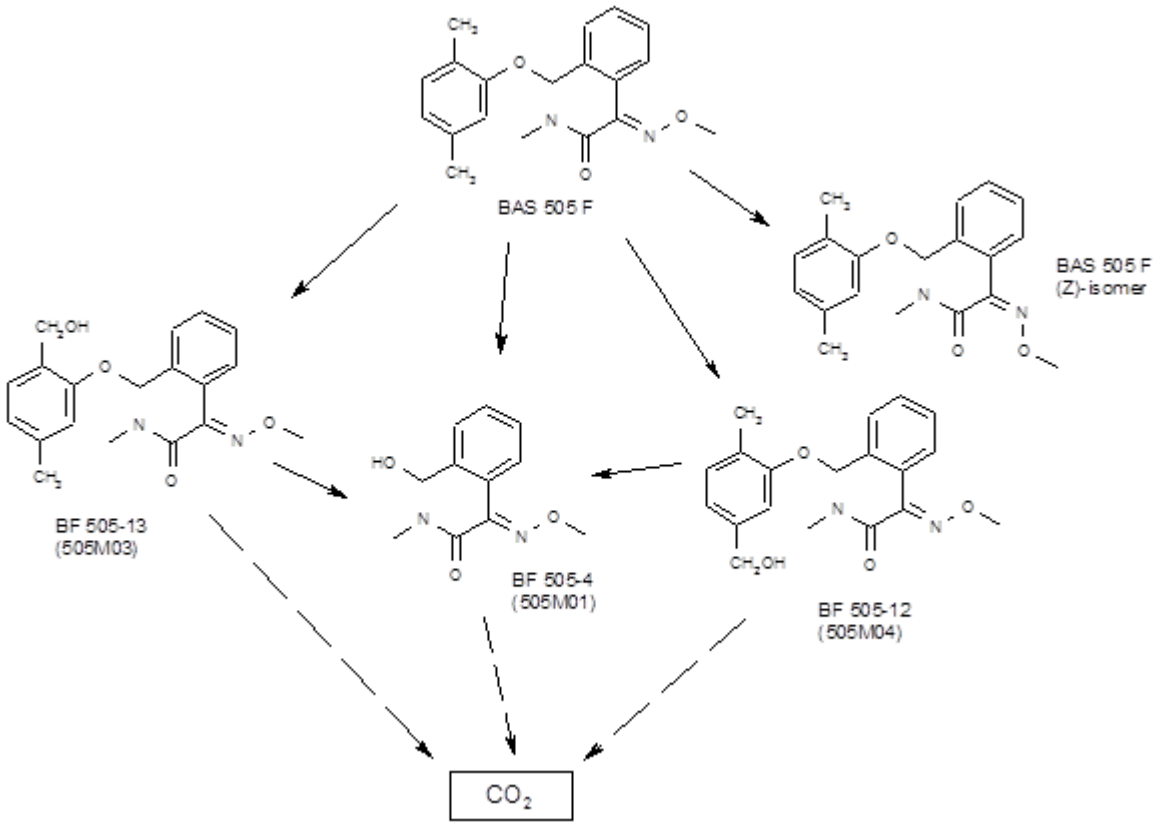
In the aqueous photolysis study performed in sterile buffer, dimoxystrobin degraded slowly with half-lives of 62 and 64 days (2 radiolabels). One cleavage product (505M01) was detected in amounts of 7.8%. All other photolysis products never exceeded 4%.

Study 2: Natural water photolysis

The aqueous photolysis of dimoxystrobin in a natural surface water was studied according to US EPA Subdivision N Guideline, Series 161-2. Natural water was obtained from a pond located in Holly Springs, CA, USA. The water had a pH of 8.6, organic matter content of 6 mg/l, and a nitrate content of 4 mg/l. The study was performed with non-labelled dimoxystrobin (purity 99.9%). The test substance was dissolved in 15 ml pond water at a concentration of 2 mg/l. A thermostated glass vessel with a quartz glass cap filled with the test solution was irradiated under a xenon lamp with a light intensity of about 1664 µE/m2/sec. Wavelengths below 290 nm were filtered off. The temperature was kept at 22°C. After up to 15 days of continuous irradiation, a 1 ml sample was taken from the glass vessel and analysed by HPLC-UV for parent dimoxystrobin. One dark control sample was analysed at the same times as the irradiated sample, respectively. Study validity criteria were met.

In the natural surface water photolysis study performed with non-labelled material, the photolytic half-life of dimoxystrobin was calculated to be 14 days.

Figure 2: Proposed route of degradation of dimoxystrobin during aqueous photolysis



Overall, the results show that when reaching water, dimoxystrobin undergoes photolytic transformation forming breakdown products and polar degradation products. At the same time, adsorption to sediment takes place where dimoxystrobin is finally bound to the sediment matrix.

Overall conclusion on rapid degradability

Dimoxystrobin is considered not rapidly degradable for the purpose of classification and labelling.

11.2 Environmental transformation of metals or inorganic metals compounds

Not applicable, dimoxystrobin is an organic compound without a metal content.

11.3 Bioaccumulation

Table 39: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Bioaccumulation (BCF) study in fish (GLP): EEC 91/414; OECD 305; EPA 165-4; EEC 96/12	Bioconcentration factor: **BCF of 48 (whole fish)** + **BCF k1/k2 of 91 (whole fish)** BCF of 47 (edible tissues) BCF of 110 (viscera) Clearance time: DT_{50} (depuration half-life) = 0.5 days DT_{90} (elimination) = 1.6 days	Flow-through 35 d exposure, 14 d depuration	Anonymous, 1999 a 1999/11247

+ Mean of days 4 – 35 (plateau)

11.3.1 Estimated bioaccumulation

As relevant experimental data are available, estimations are not included.

11.3.2 Measured partition coefficient and bioaccumulation test data

An experimental aquatic study to determine the bioconcentration potential (BCF) of dimoxystrobin (purity >97%) is available following GLP and OECD Guideline 305 (Anonymous 1999a, 1999/11247). The study used radiolabelled [benzyl-U-^{14}C]- dimoxystrobin.dissolved in N,N-dimethyl formamide (DMF), a flow-through system with rainbow trout (*Oncorhynchus mykiss*) and exposure to a single concentration of test substance at 0.5 µg a.i./L (nominal). A control experiment was conducted where fish were exposed to DMF and diluent water alone. The exposure period ran for 35 days followed by a 14-day depuration period. Total radioactivity was determined in tissue and water samples. Extracts of water, edibles and inedibles were analysed by radio-HPLC and/or radio-TLC. Unchanged parent compound and degradation products were identified by chromatographic comparison with reference compounds.

The depuration half-life (CT_{50}) in whole fish was 0.5 days. The time to reach 90% depuration (CT_{90}) is 1.6 days. The bioconcentration factor calculated directly from the ratio of the ^{14}C-concentrations in water and tissue fractions (mean of Days 4 - 35) was 84 for whole fish, 47 for edible tissues and 110 for viscera. The values were in good accordance with those obtained by kinetic modelling (whole fish: 91, edibles: 49, inedibles: 120). The compound was intensively metabolized to mainly hydroxylation products and their glucuronic acid conjugates. Due to the low bioconcentration factor and the rapid excretion of the active substance from fish it is concluded that there is no risk of bioaccumulation of dimoxystrobin.

Summary and discussion of aquatic bioaccumulation

The Log P_{OW} of dimoxystrobin is 3.59. A Log P_{OW} >4 can influence the chronic classification and M-factor under CLP unless a reliable fish bioconcentration factor (BCF) is available. A Log P_{OW} > 3 also requires that an experimental fish bioconcentration study be conducted under pesticide regulations. Such a study has been submitted by the applicant of the active substance and is considered for the evaluation of the bioaccumulative potential of dimoxystrobin. A detailed summary of the study including design and methods as well as the results can be found in Annex I, chapter 4.2.1. In the following a short summary is presented.

The study used radiolabelled [benzyl-U-^{14}C]- dimoxystrobin dissolved in N,N-dimethyl formamide (DMF), a flow-through system with Rainbow Trout (*Oncorhynchus mykiss*) and exposure to a single concentration of test substance at 0.5 µg a.i./L (nominal). A control experiment was conducted where fish were exposed to DMF and diluent water alone. The exposure period ran for 35 days followed by a 14-day depuration period. Total radioactivity was determined in tissue and water samples. Extracts of water, edibles and inedibles were analysed by radio-HPLC and/or radio-TLC. Unchanged parent compound and degradation products were identified by chromatographic comparison with reference compounds. After exposure of fish to dimoxystrobin at a nominal exposure level of 0.5µg/L, a plateau was reached after 14 days. After termination of the exposure, radioactivity levels in fish tissues decreased rapidly with a half-life of ca. 0.5 days. Bioconcentration factors calculated directly from the ratio of the '4C-concentrations in water and tissue fractions (mean of Days 4 - 35) were relatively low in edibles (47) as well as in inedibles (110). The compound was intensively metabolised to mainly hydroxylation products and their glucuronic acid conjugates. Due to the low bioconcentration factor and the rapid excretion of the active substance from fish it is concluded that there is no risk of bioaccumulation of dimoxystrobin.

11.4 Acute aquatic hazard

A summary of the suitable acute aquatic toxicity studies for dimoxystrobin, as reviewed under EU Regulation 1107/2009, is presented in Table 40below. The studies have been evaluated, considered reliable and deemed suitable for hazard classification purposes by the dossier submitter. All studies below conformed to GLP certification and were valid according to the criteria of the respective test guidelines. Additional information on the studies supporting the classification of dimoxystrobin has been presented in the subsections below.

Table 40 summarises the studies which were already evaluated as part of the inclusion process of the active substance to Commission Implementing Regulation (EU) 540/2011 (EU approval process for active substances of plant protection products) and new studies for the active substance dimoxystrobin regarding its acute toxicity to all aquatic organism groups (trophic levels) relevant for classification purposes. The lowest endpoint is highlighted in bold if several endpoints are available for the same species or group. Summaries of the relevant studies triggering classification are provided below.

Table 40: Summary of relevant information on acute aquatic toxicity

Guideline	Species	Endpoint Data	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/L)[3)] [data endpoint is based upon[3]]	
Fish							
EPA 72-1	*Oncorhynchus mykiss*	Mortality	Static	96h	LC_{50}	**0.0434 mm**	Anonymous, 1998 1998/10601
EPA 72-1, EPA 850.1075	*Oncorhynchus mykiss*	Mortality	Flow-through	96h	LC_{50}	0.0444 mm	Anonymous, 2000 2000/5125
EPA 72-3(a), EPA 850.1075	*Cyprinodon variegatus*	Mortality	Flow-through	96h	LC_{50}	0.167 mm	Anonymous, 2000a 2000/5062
EPA 72-1	*Lepomis macrochirus*	Mortality	Static	96h	LC_{50}	0.0512 mm	Anonymous, 1998b 1998/10620
EPA 850.1075; EPA 72-1	*Lepomis macrochirus*	Mortality	Flow-through	96h	LC_{50}	0.0519 mm	Anonymous, 2000b 2000/5092
Aquatic invertebrates							
OECD 202	*Daphnia magna*	Immobility	Static	48h	EC_{50}	**0.0394 n**	Dohmen P., 1999 a
OECD 202	*Asellus aquaticus*	Mortality	Static	48h	LC_{50}	0.437 mm	Janson G.-M.; Dohmen G.P., 2008 a
EPA 72-3, EPA 850.1035	*Americamysis bahia*	Mortality	Flow through	48h	LC_{50} [1)]	0.0429 mm	Wyskiel D.C. et al., 2000 b
EPA 72-3, EPA 850.1025	*Crassostrea virginica* [2)]	Shell growth inhibition, mortality	Flow through	96h	EC_{50}	0.00842 mm [shell growth inhibition]	Wyskiel D.C. et al., 2000 c
Algae / aquatic plants							
OECD 201	*Pseudokirchneriella subcapitata*	Growth rate	Static	96h	E_rC_{50}	0.153 nom	Kubitza J., 1999 a
				96h	E_rC_{10}	0.0133 mm	
EPA 123-2, EPA 850.5400	*Navicula pelliculosa*	Growth rate	Static	72h	E_rC_{50}	**0.0078 mm** [+]	Wyskiel D.C. et al., 2000 d

EPA 123-2, EPA 850.5400	*Anabaena flos-aquae*	Growth rate	Static	72h	E_rC_{50}	> 2.06 mm	Wyskiel D.C. et al., 2000 e
EPA 123-2, EPA 850.5400	*Skeletonema costatum*	Growth rate	Static	72h	E_rC_{50}	> 4.31 mm [+]	Wyskiel D.C. et al., 2000 a
EPA 123-2, EPA 850.4400	*Lemna gibba*	Frond number	Static	14d	E_bC_{50}	0.149 im	Wyskiel D.C. et al., 2000 f

[+] The 72 h growth rate endpoint obtained in the 120 h alga study is used in accordance with recent EFSA Aquatic GD (2013).

1) For better comparison of the endpoint to *Daphnia* the LC_{50} at 48 h of the *A. bahia* is reported.

2) A study on the mollusc *Crassostrea virginica* is available, however for classification purposes only aquatic crustacean species are relevant according to Regulation (EC) No 1272/2008 (CLP Regulation).

3) Endpoints in **bold** are the critical endpoints for that organism group. The letters to the right of the numbers refer to what concentration of the active substance the endpoint is based on:

n – nominal mm – mean measured im – initial measured

11.4.1 Acute (short-term) toxicity to fish

Five acute (short-term) fish studies are available for the active substance dimoxystrobin (see Table 40). With exception of the study on *Cyprinodon variegatus* (Anonymous 2000a, 2000/5062), all have been evaluated on EU-level in the relevant EU documents (DAR (Draft Assessment Report 2005), DAR Addendum 2009 and EFSA conclusion (EFSA Scientific Report (2005) 46, 1-82)).

The lowest acute fish toxicity endpoint (96 h LC_{50} = 0.0434 mg/L, Anonymous 1998a, 1998/10601) is derived from the study on *Oncorhynchus mykiss* and is the key study for classification purposes. Therefore, a short summary is presented below and in greater detail in Annex I of this document.

The four studies which are no key studies for classification purposes were all performed to recent EPA guidelines under GLP and are seen as supportive information. For these studies a short summary is also presented below and in greater detail in Annex I of this document.

Anonymous 1998a, 1998/10601

In a 96-hour flow-through acute toxicity laboratory study, rainbow trout were exposed to a dilution water control, a solvent control and to dimoxystrobin at nominal concentrations of 0.01, 0.0147, 0.0215, 0.0316, 0.0464, 0.0681 and 0.1 mg a.s./L (corresponding to mean measured concentrations of 0.093, 0.0156, 0.0205, 0.0302, 0.0434, 0.0705 and 0.0898 mg a.s./L) in groups of 10 animals in glass aquaria containing. Fish were observed for survival and symptoms of toxicity directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure. Based on the nominal concentrations the median lethal concentration LC_{50} (96 h) is about 0.0464 mg a.s./L. Mortality occurred first at the concentration of 0.0464 mg a.s./L. Behavioral symptoms such as apathy and tumbling were monitored at the test concentrations 0.0464 mg a.s./L. Other substance related effects included convulsions and narcotic-like state. These were observed in the test concentrations 0.0681 mg a.s./L and 0.1 mg a.s./L before the fish died. The NOEC (96 h) is about 0.0316 mg a.s./L (nominal). Based on the mean values of the analytically detected filtrated concentrations the median lethal concentration LC_{50} (96 h) is about 0.0434 mg a.s./L.

Anonymous 2000a, 2000/5062

In a 96-hour flow-through acute toxicity laboratory study, juvenile sheepshead minnows were exposed to a dilution water control, a solvent control and to dimoxystrobin at nominal concentrations of 0.065, 0.110, 0.180, 0.300 and 0.500 mg a.s./L (corresponding to mean measured concentrations of 0.0576, 0.113, 0.189, 0.301 and 0.512 mg a.s./L) in groups of 10 animals in glass aquaria containing 15 L water. Fish were observed for survival and symptoms of toxicity directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure. The biological results are based on mean measured concentrations of the test item. After 96 hours of exposure, no mortality was observed in the dilution water control and at the lowest test item concentration of 0.0576 mg a.s./L, whereas 20%, 50%, 100% and 100% mortality was observed at test item concentrations of 0.113, 0.189, 0.301 and 0.512 mg a.s./L. In the solvent control, 5% mortality occurred. No sub-lethal effects were found in the control groups and in all test item treatments after 96 hours. The LC_{50} (96 h) of dimoxystrobin was 0.167 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.0576 mg a.s./L (mean measured).

Anonymous 2000a, 2000/5125

In a static acute toxicity laboratory study, juvenile rainbow trout were exposed to , 0.013, 0.022, 0.036, 0.06 and 0.1 mg dimoxystrobin/L (nominal) in groups of 20 animals in glass aquaria containing 15 L water with 2 replicates per concentration. Fish were observed for survival and symptoms of toxicity within 24, 48, 72 and 96 hours after start of exposure. The biological results are based on mean measured concentrations. After 96 hours of exposure no mortality and toxic effects were observed in the control and the solvent control and at concentrations of up to and including 0.022 mg dimoxystrobin/L, whereas 5%, 95% and 100% mortality were observed at the three highest test item concentrations of 0.036, 0.06 and 0.1 mg/L, respectively. Surviving fish showed sub-lethal effects (*e.g.* loss of equilibrium, erratic swimming, lethargy). The LC_{50}

(96 h) for dimoxystrobin was determined to be 0.0444 mg/L based on mean measured concentrations. The NOEC (96 h) was 0.0218 mg/L (nominal).

Anonymous 1998b, 1998/10620

In a static acute toxicity laboratory study, juvenile bluegills (approx. 11 months old) were exposed to 0.0147, 0.0215, 0.0316, 0.0464, 0.0681 and 0.1 mg dimoxystrobin/L (nominal) in groups of 10 animals in glass aquaria containing 100 L water with 2 replicates per concentration. Fish were observed for survival and symptoms of toxicity within 1 hour after start of exposure and 4, 24, 48, 72 and 96 hours after start of exposure. After 96 hours of exposure no mortality and toxic effects were observed in the control and at concentrations of up to and including 0.0464 mg dimoxystrobin/L, whereas 100% mortality was observed at the 0.0681 mg/L and 80% mortality at the highest concentrations of 0.1 mg/L. At a concentration of 0.1 mg dimoxystrobin/L surviving fish showed sub-lethal effects (*e.g.* apathy). The LC_{50} (96 h) for dimoxystrobin was determined to be 0.0512 mg/L based on mean measured values. The NOEC (96 h) was 0.0424 mg/L (mean measured).

Anonymous 2000b, 2000/5092

In a static acute toxicity laboratory study, juvenile bluegills were exposed to 0.013, 0.022, 0.036, 0.06 and 0.1 mg dimoxystrobin/L (nominal) in groups of 20 animals in glass aquaria containing 15 L water with 2 replicates per concentration. Fish were observed for survival and symptoms of toxicity at 24, 48, 72 and 96 hours after start of exposure. No mortality of fish was observed in the control. In the solvent control a survival of 95% occurred. No control or solvent control sublethal effects were noted during the exposure period. Fish exposed to 0.0579 mg dimoxystrobin/L exhibited a loss of equilibrium at 24 and 48 hours, lethargy or a loss of equilibrium at 72 hours and lethargy at 96 hours. Fish exposed to 0.0981 mg a.s./L exhibited a loss of equilibrium and a change of coloration at 24 hours. The LC_{50} (96 h) for dimoxystrobin was determined to be 0.052 mg/L based on mean measured values. The NOEC (96 h) was 0.036 mg/L (mean measured).

11.4.2 Acute (short-term) toxicity to aquatic invertebrates

Acute (short-term) studies on aquatic crustaceans are available for *Daphnia magna*, *Asellus aquaticus* and *Americamysis bahia*. Additionally, a study on the mollusc *Crassostrea virginica* is available, however for classification purposes only aquatic crustacean species are relevant according to Regulation (EC) No 1272/2008 (CLP Regulation).

The lowest endpoint for aquatic crustaceans is derived from the study on *Daphnia magna* (48 h EC_{50} = 0.0394 mg/L, Dohmen P.(1999a)) and is the key study for classification purposes. Therefore, a short summary is presented below and in greater detail in Annex I of this document.

The study on *Asellus aquaticus* was performed according to recent OECD guideline under GLP and is summarized in the DAR Addendum 2009 and is seen as supportive information. However, for all studies which were not considered key studies for classification purposes a short summary is also presented below and in greater detail in Annex I of this document.

Dohmen P.(1999a),

In a 48-hour static acute toxicity laboratory study, the effect of dimoxystrobin on water flea neonates was investigated. Neonates less than 24 hours old were exposed to nominal concentrations of 2, 4, 8, 16, 32 and 64 µg a.s./L (nominal). Additionally, a dilution water control was set up. Daphnids were exposed in 4 replicates per concentration, containing 5 daphnids each. The daphnids were observed for immobility 24 and 48 hours after start of exposure. The following biological results are based on nominal concentrations. Significant immobility of the daphnids was only observed at the two highest concentrations with 20% immobility at 32 µg a.s./L and complete immobility at 64 µg a.s./L after 48 hours; at 16 µg a.s./L one immobile daphnid was observed. The EC_{50} of dimoxystrobin was determined to be 39.4 µg a.s./L, the NOEC was 8 µg a.s./L (nominal).

Wyskiel D.C. et al.(2000b)

In a 96-hour flow-through acute toxicity laboratory study, saltwater mysids were exposed to a dilution water control, a solvent control and to nominal concentrations of 0.0065, 0.011, 0.018, 0.030 and 0.050 mg dimoxystrobin/L (nominal) (corresponding to mean measured concentrations of 0.00767, 0.0111, 0.018, 0.0299 and 0.0497 mg a.s./L) in two replicates per treatment containing 10 mysids each. Saltwater mysids were observed for survival and symptoms of toxicity directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure. The biological results are based on mean measured concentrations of the test item (measured over the 96 h study period). After 48 hours of exposure, no mortality was observed in the control, the solvent control and at concentrations of up to and including 0.018 mg/L, whereas 10% and 70% mortality was observed at the two highest test item concentrations of 0.0229 and 0.0497 mg a.s./L, respectively. After 48 hours, sub-lethal effects, observed as lethargy and/or erratic swimming, were noted at the two highest tested concentrations of dimoxystrobin. The LC_{50} (48 h) for dimoxystrobin was determined to be 0.0429 mg a.s./L, based on mean measured concentrations.

Wyskiel D.C. et al. (2000c)

In a 96-hour acute toxicity laboratory study the effect of dimoxystrobin on shell deposition of eastern oysters was investigated under flow-through conditions. The eastern oysters were exposed to a dilution water control, a solvent control and to dimoxystrobin at nominal concentrations of 0.0033, 0.0055, 0.0090, 0.015 and 0.025 mg a.s./L (corresponding to mean measured concentrations of 0.00301, 0.00514, 0.00814, 0.0141 and 0.0237 mg a.s./L) in groups of 10 oysters per replicate with two replicates per treatment. Eastern oysters were observed for survival and symptoms of toxicity daily during the exposure period. Measurements of shell deposition for each oyster were made after 96 hours. The biological results are based on mean measured concentrations of the test item. After 96 hours, no mortality occurred at test item concentrations of up to and including 0.00514 mg a.s./L, whereas 5%, 10% and 30% mortality was observed at 0.00814, 0.0141 and 0.0237 mg a.s./L, respectively. After 96 hours, sub-lethal effects, observed as oysters exhibited a delayed reaction to gentle prodding (slowly closing shells) at the two highest concentrations of dimoxystrobin. Statistically significant inhibition of shell growth compared to the pooled control / solvent control was observed at the four highest tested concentrations. The EC_{50} (96 h) for dimoxystrobin was 0.00842 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.00301 mg a.s./L (mean measured).

Janson G.-M., Dohmen G.P. (2008a)

The acute toxicity of dimoxystrobin to *Asellus aquaticus* was investigated in a 96 h static toxicity study. 10 replicates per treatment group were treated with nominal test concentrations of 0.030, 0.060, 0.120, 0.240 and 0.480 mg dimoxystrobin/L, plus a control and a solvent control. Animals were observed for mortality and other symptoms 24, 48, 72 and 96 hours after start of exposure. The biological results are based on nominal concentrations. After 48 h of exposure, no mortality of the test organisms were observed in the controls and at up to and including the test item concentration of 0.120 mg a.s./L. At the two highest tested concentrations of 0.240 and 0.480 mg a.s./L, 30% and 50% mortality occurred, respectively. The effects in all test item treatments were not statistically significantly different compared to the controls. No sub-lethal impact of the test substance was observed. The LC_{50} (48 h) of dimoxystrobin was determined to be 0.437 mg a.s./L, based on nominal concentrations.

11.4.3 Acute (short-term) toxicity to algae or other aquatic plants

Four short-term studies on algae are available and one additional study with the aquatic macrophyte *Lemna gibba*. The lowest endpoint is derived from the study on *Navicula pelliculosa* and is the relevant study for classification purposes. Therefore, a short summary is presented below and in greater detail in Annex I of this document.

The additional algae and aquatic plant studies were submitted for Annex I renewal of dimoxystrobin and have not been evaluated previously on EU level, therefore a short summary is also presented below. A more detailed summary is provided in Annex I of this document. They are seen as supportive information and have all been conducted according to recent OECD and EPA guidelines under GLP.

Wyskiel D.C. et al., (2000d)

In a 120-hour static toxicity laboratory study, the effect of dimoxystrobin on the growth of the freshwater diatom *Navicula pelliculosa* was investigated. The following nominal concentrations were applied: 0.00086, 0.0017, 0.0034, 0.0065 and 0.013 mg dimoxystrobin/L (corresponding to mean measured concentrations of 0.00122, 0.00177, 0.00359, 0.00607 and 0.0138 mg a.s./L). Additionally, a solvent control (dimethylformamide) and a dilution water control were set up. Assessment of growth was conducted 24, 48, 72, 96 and 120 h after test initiation. The biological results are based on mean measured concentrations of

the test item. Statistically significant differences compared to the pooled control were observed at the four highest test item concentrations after exposure over 120 hours. After 72 hours, the E_rC_{50} and E_bC_{50} values were determined to be 0.0078 mg a.s./L and 0.0025 mg a.s./L, respectively (mean measured).

Wyskiel D.C. et al. (2000 e)

In a 120-hour static toxicity laboratory study, the effect of dimoxystrobin on growth of the blue-green alga *Anabaena flos-aquae* was investigated. The following nominal concentrations were applied: 0.13, 0.25, 0.50, 1.0 and 2.0 mg dimoxystrobin/L (corresponding to mean measured concentrations of 0.133, 0.257, 0.524, 1.07 and 2.06 mg a.s./L). Additionally, a solvent control (dimethylformamide) and a dilution water control were set up. Assessment of growth was conducted 24 h, 48 h, 72 h, 96 h and 120 h after test initiation. The biological results are based on mean measured concentrations of the test item. Statistically significant differences compared to the pooled control were observed at the four highest test item concentrations after exposure over 120 hours. The 72 h and 120 h E_rC_{50} values for dimoxystrobin was determined to be both > 2.06 mg a.s./L based on mean measured concentrations. The 72 h and 120 h E_bC_{50} values were 0.960 and 1.13 mg a.s./L, respectively (mean measured).

Wyskiel D.C. et al. (2000 a)

In a 120-h static toxicity laboratory study, the effect of dimoxystrobin on the growth of the marine diatom *Skeletonema costatum* was investigated. The following nominal concentrations were applied: 0.33, 0.66, 1.3, 2.5 and 5.0 mg dimoxystrobin/L, corresponding to mean measured concentrations of 0.330, 0.657, 1.29, 2.32 and 4.31 mg a.s./L. Additionally, a solvent control (dimethylformamide) and a dilution water control were set up. Assessment of growth was conducted directly after start of exposure and after 24, 48, 72, 96 and 120 h after test initiation. The biological results are based on mean measured concentrations of the test item. After 120 hours of exposure statistically significant differences compared to the pooled control were observed for growth rate at the highest test item concentration. The 72 h and 120 h E_rC_{50} were determined to be both > 4.31 mg a.s./L based on mean measured concentrations. The 72 h and 120 h E_bC_{50} values were 1.28 mg a.s./L and > 4.31 mg a.s./L, respectively (mean measured).

Kubitza J. (1999 a)

In a 96-h static toxicity laboratory study, the effect of dimoxystrobin on the growth of the unicellular fresh water green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 4, 8, 16, 32, 64, 128, 256 µg a.s./L. Additionally, a dilution water control was set up. Assessment of growth was conducted 48, 72 and 96 h after test initiation. The results are based on nominal concentrations. No morphological effects were observed at concentration up to 16 µg a.s./L. At the concentrations 32 µg a.s./L to 256 µg a.s./L a few cells exhibited morphological changes (the algal cells appeared rounder than those in the control). A test prolongation demonstrated quick recovery showing that the effects are algistatic rather than algicidal. The E_rC_{50} of dimoxystrobin was determined to be 152.6 µg a.s./L, the E_rC_{10} was 13.3 µg a.s./L. Effects can be rated algistatic rather than algicidal.

Wyskiel D.C. et al. (2000 f)

In a 14-day static toxicity laboratory study, the effect of dimoxystrobin on the growth of the duckweed *Lemna gibba* was investigated. The following nominal concentrations were applied: 0.033, 0.065, 0.13, 0.25 and 0.50 mg dimoxystrobin/L (corresponding to initial measured concentrations of 0.0335, 0.0634, 0.132, 0.247 and 0.444 mg a.s./L). Additionally, a solvent control (dimethylformamide) and a dilution water control were set up. Assessment of plant growth and other effects was conducted 2, 5, 7, 9, 12 and 14 days after test

initiation. Percent growth inhibition relative to the control was calculated for each test concentration based upon biomass for the parameter frond number. Dry weight was determined at test termination. The biological results are based on initial measured concentrations of the test item. The duckweed population in the control vessels showed sufficient growth. At the end of the test, chlorotic fronds were observed in the control, the solvent control and at the test item concentrations of 0.0335, 0.0634 and 0.444 mg a.s./L. Statistically significant effects on the number of non-chlorotic fronds and plant dry weight compared to the pooled control were observed at the four highest tested concentrations and at the three highest tested concentrations, respectively. The E_bC_{50} values of dimoxystrobin were determined to be 0.149 mg a.s./L based on frond number and 0.226 mg a.s./L based on dry weight (initial measured).

11.4.4 Acute (short-term) toxicity other aquatic organisms

No relevant data available.

11.5 Long-term aquatic hazard

Table 41 summarizes the relevant endpoints for the active substance dimoxystrobin regarding its chronic toxicity to all aquatic organism groups (trophic levels) relevant for classification purposes. Brief summaries of the relevant studies triggering classification are provided below. More detailed summaries are provided in Annex I.

Table 41: Summary of relevant information on chronic aquatic toxicity

Guideline	Species	Endpoint Data	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/L)[2] [data endpoint is based upon[3]]	
Fish							
OECD 204 [3]	*Oncorhynchus mykiss*	Mortality, growth, sublethal effects	Flow-through	28d	NOEC	0.010 n	Anonymous, 1999a 1999/10311
OECD 210, EPA 72-4	*Oncorhynchus mykiss*	Growth, sublethal effects	Early life-stage test Flow-through	97d	NOAEC	**0.0010 n**	Anonymous, 1999 1999/10521
EPA 850.1400, EPA 72-4	*Pimephales promelas*	Mortality	Early life-stage test Flow-through	36d	NOEC	0.016 mm	Anonymous, 2000c 2000/5156
Aquatic invertebrates							
OECD 202; EPA 660/3-75-009	*Daphnia magna*	Reproduction	Semi-static	21d	NOEC	**0.0125 n**	Jatzek H.-J., 2000 a
Algae / aquatic plants							
OECD 201	*Pseudokirchneriella subcapitata*	Growth rate	Static	96h	E_rC_{50}	0.153 n	Kubitza J., 1999 a
				96h	E_rC_{10}	0.0133 mm	
EPA 132-2, EPA 850.5400	*Navicula pelliculosa*	Growth rate	Static	120h	NOE_rC	**0.00122 mm**	Wyskiel D.C. et al., 2000 d
EPA 132-2, EPA 850.5400	*Anabaena flos-aquae*	Growth rate	Static	120h	NOE_rC	0.133 mm	Wyskiel D.C. et al., 2000 e

Guideline	Species	Endpoint Data	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/L)[2] [data endpoint is based upon[3]]	
EPA 123-2, EPA 850.5400	*Skeletonema costatum*	Growth rate	Static	120h	NOE_rC	2.32 mm	Wyskiel D.C. et al., 2000 a
EPA 123-2, EPA 850.4400	*Lemna gibba*	Frond number	Static	14d	NOE_bC	0.0335 im	Wyskiel D.C. et al., 2000 f
Sediment-dwelling organisms							
BBA proposal 1995	*Chironomus riparius* [1]	Emergence rate	flow-through, spiked water	28d	NOEC	**0.010 n**	Dohmen G.P., 2001 a

[1] A study on the aquatic insect *Chironomus riparius* is available, however for classification purposes only aquatic crustacean species are relevant according to Regulation (EC) No 1272/2008 (CLP Regulation). A detailed summary can be found in Annex I, chapter 4.5.

[2] Endpoints in **bold** are the critical endpoints for that organism group.

[3] Study is not considered a suitable long-term study according to the Guidance on Information Requirements and Chemical Safety Assessment (Chapter R.7b: Endpoint specific guidance, version 4.0, June 2017) as it does not evaluate effects off sensitive life stages. However, it is presented as additional information. A detailed summary can be found in Annex I, chapter 4.5.

The letters to the right of the numbers refer to what concentration of the active substance the endpoint is based on:
n – nominal mm – mean measured im – initial measured

11.5.1 Chronic toxicity to fish

Three chronic fish studies are available with the active substance dimoxystrobin which are summarized in the relevant EU documents (DAR 2005, DAR Addendum 2009) and are assessed in the EFSA conclusion on dimoxystrobin.

The lowest chronic fish toxicity endpoint is derived from a ELS study on rainbow trout and is the relevant study for classification purposes. Therefore, a short summary is presented below and in greater detail in Annex I of this document. The additional chronic fish study was performed to recent EPA guidelines under GLP and is seen as supportive information. A short summary is presented below and in greater detail in Annex I of this document.

Furthermore, a 28 d study on rainbow trout was performed. The study is not considered a suitable long-term study according to the Guidance on Information Requirements and Chemical Safety Assessment (Chapter R.7b: Endpoint specific guidance, version 4.0, June 2017) as it does not evaluate effects off sensitive life stages. However, it is presented as additional information. A short summary is presented below and in greater detailed in Annex I, chapter 4.5.

Anonymous 1999b, 1999/10521

The chronic toxicity of dimoxystrobin to Rainbow trout (*Oncorhynchus mykiss*) embryos and fry was investigated in a 97-day early life-stage test under flow-through conditions. Embryos were exposed to a dilution water control and to dimoxystrobin at nominal concentrations of 0.000316, 0.001, 0.00316, 0.01 and 0.0316 mg a.s./L. Hatchability, survival rate and behavior of sheepshead minnow embryos and fry were

assessed throughout the study. Individual fish lengths and weights were measured at test termination. The biological results are based on nominal concentrations. In the highest test concentration 0.0316 mg a.s./L the embryos were killed nearly quantitatively at hatching. In the second highest concentration (0.01 mg a.s./L) the survival of the young fish was moderately impaired. There were no sublethal effects caused by the test compound in the two lowest concentration (0.000316 and 0.001 mg a.s./L).

In the higher concentrations 0.00316 and 0.01 mg a.s./L there were clear compound-related sublethal effects such as abnormalities of the tail and/or body, swimming in circles as sequela of abnormal tail, narcotic-like state, retarded resorption of yolk sac and untypically extended yolk sac. The development of the mean body weight measured at the end of the study was impaired in the two highest dose groups with surviving fish. The mean body length at (97 d) was lower in the 3 highest concentrations with surviving, however, only to a very minor extent - 5% reduction - at 0.001 mg/L. Thus, there were apparent reductions in mean body weight (not statistically significant) and body length (statistically significant) at 0.001 mg/L. However, at the same time the numbers of surviving fish was higher in the 0.001 mg/L treatment. If the higher numbers of fish are taken into consideration and the total biomass or length per replicate (i.e. the sum of the fish weights and length per replicate) is used than it becomes clear that there is no statistically or biologically significant difference to the control in the 0.001 mg/L treatment. The NOEC is thus (deviating from the report to the study) established at 0.001 mg/L, the LOEC with small but significant effects on biomass is 0.00316 mg/L. A overall NOAEC of 0.001 mg a.s./L and an overall LOAEC of 0.00316 mg a.s./L has been determined.

Anonymous 2000c, 2000/5156

In a 36 d flow-through toxicity study, embryos of *Pimephales promelas* were exposted to 2.2, 4.2, 8.0, 16 and 32 µg dimoxystrobin/L. Four replicates of 10 embryos per test vessel and per concentration were set up. The study was terminated on day 36, 32 days after completion of hatch and young fish survival, sublethal effects as well as effects on weight and length were assessed. There was at least 95% hatch in each replicate control and solvent control vessel, the hatch in each control chamber was less than 1.6 times the hatch in the other control chambers, and the number of live, normal control and solvent control fish at 36 days of exposure (32 days post hatch) was at least 70% in each vessel. Control and solvent control fish had an average wet weight (blotted) of 89.3 mg and 92.3 mg, and average dry weight of 26.5 mg and 27.1 mg and an average total length of 24.6 mm and 24.1 mm, respectively, at the end of the test. The relative standard deviation of the weights of surviving fish in the control test chamber was less than 40%. Maximum control loading rate during the toxicity test was approximately 0.13 g/L at any time and 0.017 g/L/24 hours. Sublethal effects, observed as one fish exhibited a deformed tail, were noted at 32 µg/L on days 18 through 21. This effect was not observed at any other time during the test. No significant sublethal effects (other than size differences) were observed at the lower concentrations of dimoxystrobin at any time. The most sensitive measured biological endpoints were the survival of fish (particularly during time of hatching and shortly afterwards). A slight, but not statistically significant reduction in biomass was observed at the highest concentrations. The NOEC of 16 µg a.s./L has been determined.

Anonymous 1999a, 1999/10311

In a 28 day flow-through toxicity study, juvenile rainbow trout (approx. 6 months old) were exposed to 0.000316, 0.001, 0.00316, 0.01 and 0.0316 mg dimoxystrobin/L (nominal) in groups of 20 animals in glass aquaria containing 60 L water. Fish were observed for survival and symptoms of toxicity daily. Mortality occurred only in the highest substance concentration 0.0316 mg dimoxystrobin/L. It started on day 1 (10%) and increased to 55% on day 8. No further increase in mortality until day 28 (end of study) was noted. Compound-related toxic signs were observed only in the highest concentration (0.0316 mg dimoxystrobin/L) starting on day 26 in form of reduced or no food uptake and swimming near the bottom. The mean body weight and the mean body length at the end of the study were not statistically different from the control group. In the highest concentration there was however a marginal decrease of body weight gain (3.4%) and

decrease in body length (3%) compared to the control. The NOAEC (28 d) for dimoxystrobin was determined to be 0.01 mg/L based on nominal concentrations.

11.5.2 Chronic toxicity to aquatic invertebrates

A chronic study on *Daphnia magna* is available with the active substance dimoxystrobin. The study has already been evaluated on EU-level. As it is the relevant study for classification purposes a short summary is presented below and in greater detail in Annex I of this document.

Jatzek H.-J. (2000 a)

In a 21-day semi-static toxicity test, effects of dimoxystrobin to water fleas (*Daphnia magna*) were examined. Neonates less than 24 hours old were exposed to nominal concentrations of 1.56, 3.13, 6.25, 12.5, 25 and 50 µg a.s./L. Additionally, a dilution water control and a solvent control were set up. All treatment groups and the controls consisted of 10 replicates containing one daphnid. Assessment of parent mortality and reproduction was conducted daily. The biological results are based on nominal concentrations. Parent mortality was observed only at 50 µg a.s./L, the highest concentration tested. The mean number of offspring per parent in the control and the solvent control was 95.7 and 144.6, respectively. No offspring was produced in the 50 µg a.s./L test concentration. A slight but statistically non-significant reduction in the number of offspring was observed at 25 µg a.s./L. First juveniles were observed at day 8 in each treatment. At 25 µg a.s./L some aborted eggs were observed, in addition a significant number of dead young were found in this variant. The NOEC of dimoxystrobin was 12.5 µg a.s./L (nominal).

11.5.3 Chronic toxicity to algae or other aquatic plants

Please see study summaries for algae and aquatic plants provided under 11.4.2 and in Annex I (chapter 4.3).

11.5.4 Chronic toxicity to other aquatic organisms

Dohmen G.P. (2001 a)

Groups of 25 individuals of *Chironomus riparius* (4 replicates) were exposed to five test concentrations (3, 10, 32, 100 and 316 µg a.s./L) of dimoxystrobin for 28 days under static conditions in a water-sediment system. A control group exposed to test water without test item as well as a solvent control were run concurrently. The invertebrates were observed daily behavioral differences and emergence of female and male midges. The emergence rates were generally quite high. More than 70% - the required minimum for the untreated controls - of the chironomids emerged in all but the highest test concentration. Statistically significant effects were obtained at the three highest test concentrations. However, also the highest test concentration of 316 µg/L caused a reduction in emergence of less than 50% (39%). The EC_{50} for emergence rate is thus > 316 µg/L. There were no significant treatment effects on the development rate although the onset of emergence was 1-2 days later at the highest test concentration. The NOEC was determined to be 10 µg/L, the LOEC was 32 µg/L and the LC_{50} was > 316 µg/L.

11.6 Comparison with the CLP criteria

11.6.1 Acute aquatic hazard

Aquatic acute toxicity data are available for fish, invertebrates, algae and aquatic plants. The most acutely sensitive trophic group are algae with a 72-hour E_rC_{50} value for *Navicula pelliculosa* of 0.0078 mg/L. On the basis of this acute algae endpoint being in the range 0.001 mg/L < EC_{50} ≤ 0.01 mg/L, dimoxystrobin should be classified as Aquatic Acute 1 (H400) with an acute M factor of 100.

11.6.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

The biodegradation degree of dimoxystrobin in the manometric respirometry test was 0 - 10% after an exposure period of 28 days. Furthermore, dimoxystrobin is hydrolytically stable and no significant degradation due to aerobic mineralisation in surface water was observed. Consequently, dimoxystrobin has to be classified as 'not rapidly degradable'. This is further confirmed by two water sediment studies evaluating degradation and distribution under laboratory and outdoor conditions as well as two studies on photochemical degradation in water systems.

The log P_{ow} of the active substance dimoxystrobin was determined to be 3.59 and therefore a bioaccumulation study in fish was performed (Anonymous 1999a, 1999/11247). After exposure of fish to dimoxystrobin at a nominal exposure level of 0.5 µg a.s./L, a plateau was reached after 14 days. The bioconcentration factor calculated directly from the ratio of the ^{14}C-concentrations in water and tissue fractions (mean of days 4 - 35) was 84 for whole fish, 47 for edible tissues and 110 for viscera. The depuration half-life in whole fish was 0.5 days. Accordingly, the time to reach 90% depuration is 1.6 days. The compound was intensively metabolized to mainly hydroxylation products and their glucuronic acid conjugates. Due to the low bioconcentration factor and the rapid excretion of the active substance from fish it is concluded that there is low potential for bioaccumulation of dimoxystrobin.

Aquatic chronic/long-term toxicity data are also available for fish, invertebrates, algae and aquatic plants, with fish being the most sensitive species towards dimoxystrobin (97 d ELS NOEAEC = 0.0010 mg/L with *Oncorhynchus mykiss*). Evaluation of this data and considering the information of degradability of the substance leads to the conclusion that dimoxystrobin should be classified as Aquatic Chronic 1 (H410). Based on 0.0001 < NOEC ≤ 0.001 mg/L, a chronic M factor of 100 is obtained.

11.7 Conclusion on classification and labelling for environmental hazards

Aquatic Acute 1; H400: Very toxic to aquatic life

Acute M-factor = 100

Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Chronic M-factor = 100

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Dimoxystrobin has a current entry in Annex VI of CLP as Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410) with an M-factor of 10.

Dimoxystrobin, being a fungicidal active substance, has gone through the following regulatory processes:

(i) an initial risk assessment provided by the Rapporteur Member State United Kingdom (DAR published in 2005)
(ii) the current CLH process
(iii) a renewal of the approval of the active substance (RAR prepared according to the Commission Regulation (EC) No 1107/2009 by Hungary and Ireland on August 2017)

Additional information was provided by the PPP Applicant during the renewal of the approval of the active substance dimoxystrobin that included updates of already reviewed studies. These studies have already been evaluated within the Annex I inclusion of dimoxystrobin and were provided in the EU Review documents of dimoxystrobin (Draft Assessment Report (DAR), Vol. 3, B.9, 2005; EFSA Scientific Report (2005) 46, 1-82; Renewal Assessment Report (2017), Volume 3–B.8 (AS) & B.9 (AS)). The information included statistical re-evaluations, elaborations on study validity and missing analytical tables, update of endpoints and the inclusion of ECx re-calculations and relevant study summaries. These data were assessed by RAC for the preparation of this opinion.

Biodegradation

One ready biodegradability test following OECD TG 301F (biochemical oxygen demand) (Werner, 1999) was available. The degree of biodegradation (% biological oxygen demand/theoretical oxygen demand) over the 28-day test duration was in the range 0-10%. In a surface water simulation biodegradation test (OECD TG 309), the mineralisation of dimoxystrobin was below 1% after 59 days of incubation (Yeomans, 2014). Two water/sediment studies were available. In the laboratory aerobic water/sediment study investigating two systems (A=pond and B=pond-like side arm river), the DT_{50} was calculated after kinetic evaluation from Budde (2015) to be 298 and 835 days, respectively (Ebert, 2000). An outdoor aerobic sediment/water study was also reported and the resulting calculated DT_{50} after the kinetic evaluation from Budde (2015) was 27 days (Fendt, 2001).

Hydrolysis

The hydrolytic stability of dimoxystrobin was studied at 25°C (pH 5,7 and 9) and 50°C (pH 4,7 and 9). The results of the hydrolysis study indicate that dimoxystrobin was stable at all pH-values (McKenna & Baucom, 1997).

Photolysis

The aqueous photolysis of dimoxystrobin was investigated in two studies using sterile buffer and natural surface water. Dimoxystrobin photodegraded slowly in the sterile water with DT_{50}s of 62 and 64 days (2 radiolabels) (Singh, 1998, kinetic evaluation Budde, 2014). Faster photolysis was observed in the natural water study with a DT_{50} of 14 days (Goetz & Moss, 1998, kinetic evaluation Budde, 2014).

Conclusion on degradation

Dimoxystrobin was not considered rapidly degradable for the purpose of classification and labelling by the DS, namely biotic and abiotic degradation in the aquatic environment did not exceed a level of 70% or above. This was based on the available ready biodegradability test, the data from the hydrolysis studies and the data from water/sediment studies showing limited degradation.

Bioaccumulation

The bioaccumulation potential of dimoxystrobin was considered to be low based on a measured $BCF_{whole\ fish}$ of 84 L/kg for *Oncorhynchus mykiss* (Anonymous, 1999a) and a Log P_{OW} of Dimoxystrobin which is 3.59. The lipid normalised kinetic bioconcentration factor BCF_{KL} was re-calculated to be BCF_{KL} = 91 L/kg and it was presented in additional information provided by the PPP Applicant during the renewal of the approval of the active substance dimoxystrobin. The BCF was normalised to 6% lipid content, as a worst-case value. The mean lipid content in whole fish was reported to be 5.1%. This is a GLP study and fulfils all the validity criteria of OECD TG 305. The DS considers dimoxystrobin to have low potential for bioaccumulation based on available data, as the experimental BCF value did not exceed the cut-off value of 500 L/kg and the log K_{OW} was below 4.

Aquatic Toxicity

Aquatic toxicity tests for both acute and chronic aquatic toxicity are available for all three trophic levels. Only reliable studies from each trophic level with the most conservative endpoint are summarised in the table below for acute and chronic aquatic toxicity. Based on the additional data provided by the PPP Applicant, some endpoint values may have been re-calculated and these updated values are shown in the table 1 below, where relevant. The re-calculation of toxicity values has no impact on the proposed classification. Dimoxystrobin's major metabolites and formulations are shown to be of lesser toxicity based on data provided in the RAR (2017), DAR (2005), and additional information provided by the PPP Applicant and, thus, were not evaluated further for classification purposes.

***Table**: Reliable studies from each trophic level with the most conservative endpoint for acute and chronic toxicity. Endpoints in bold are the key critical endpoints for classification purposes*.

Acute toxicity				
Species	**Method**	**Endpoint (Effect Observed)**	**Toxicity value (mg a.s./L)**	**Reference (as in CLH Report unless otherwise stated)**
Fish				
Oncorhynchus mykiss	EPA 72-1	LC_{50} (96h), (Mortality)	0.0434 mm	Anonymous, 1998a

Oncorhynchus mykiss	EPA 72-3(a), EPA 850.1075	LC_{50} (96h) , (Mortality)	0.0465 (1) nm	Anonymous, 2000
Invertebrates				
Daphnia magna	OECD TG 202	EC_{50} (48h) (Immobility)	0.0394 nm	Dohmen P., 1999a
Americamysis bahia	EPA 72-3(b), EPA 850.1035	LC_{50} (48h) , (Mortality) EC_{50} (96h), (Mortality)	0.0429 mm 0.0272 mm	Wyskiel D.C. *et al.*, 2000b
Algae				
Navicula pelliculosa	EPA 123-2, EPA 850.5400	E_rC_{50} (72h), (Growth rate)	**0.0078 mm**	Wyskiel D.C. *et al.*, 2000d
Pseudokirchneriella subcapitata	OECD TG 201	E_rC_{50} (72h), (Growth rate)	0.1526 nm	Kubitza J. 1999
Chronic toxicity				
Species	**Method**	**Endpoint (Effect Observed)**	**Toxicity value (mg a.s./L)**	**Reference (as in CLH Report unless otherwise stated)**
Fish				
Oncorhynchus mykiss	OECD TG 210, EPA 72-4 (a)	NOEC, (Growth, sublethal effects)	**0.001 (1) nm**	Anonymous, 1999a
Pimephales promelas	OECD TG 210, EPA 72-4 (a)	NOEC, (Mortality)	0.016 nm	Anonymous, 2000
Oncorhynchus mykiss (2)	OECD TG 210, EPA 72-4, EPA 850.1400	NOEC, (Growth, sub-lethal effects)	0.012 nm (Peak Conc)	Anonymous, 2008a (as in RAR 2017)
Invertebrates				
Daphnia magna	EEC XI/691/86, DIN 38412 (Entwurf 1981), OECD TG 202, EPA 660/3-75-009	NOEC, (Reproduction)	0.0125 nm	Jatzek H.-J., 2000 a
Algae				
Navicula pelliculosa	EPA 123-2, EPA 850.5400	NOE_rC (120h) (Growth rate)	0.00122 (3) mm	Wyskiel D.C. *et al.*, 2000 d
Pseudokirchneriella subcapitata	OECD TG 201	E_rC_{10} (72h) (Growth rate)	**0.0035 (1) nm**	Kubitza J. 1999

(1) Data presented as the re-calculated values provided by the applicant as part of a recent Request for Additional Information, the Applicant submitted new information to EFSA.

(2) The study was available in RAR 2017 but was not included in CLH Report. Study results do not impact classification, detailed explanation is provided in the Supplemental Information, below. It is shown in the table for completeness.

(3) The OECD TG 201 guideline validity criteria for the study were confirmed to for the 72h duration alone thus this NOE_rC (120h) value cannot be used for classification purposes.

nm= nominal concentrations

mm=measured concentrations

Acute aquatic toxicity

Data is available for all three trophic levels (fish, crustacean, algae/aquatic plants). Five fish studies, three invertebrate studies, and four algae studies were evaluated in the CLH report as reliable and valid for classification purposes. Reliable studies for each trophic level with the most conservative endpoints are shown in the Table above. The lowest effects endpoint is a 72h E_rC_{50} value of 0.0078 mg/L (measured concentration) derived from a study on the algae, *Navicula pelliculosa*. Based on this endpoint, the DS proposes a classification of Aquatic Acute 1 (H400) with an acute M factor of 100.

Chronic aquatic toxicity

Data is also available for all three trophic levels (fish, crustacean, algae/aquatic plants). Three fish studies, one invertebrate study and four algae studies were evaluated in the CLH report as reliable and valid for classification purposes. Reliable studies for each trophic level with the most conservative endpoints are shown in the Table. A study by Anonymous (2008a) that was available in the RAR (2017) but was not included in the CLH Report is also shown in the Table above. The study results do not impact classification. The executive summary of the study and detailed explanation on the assessment of the study is provided in the Supplemental Information, below. It is shown in the Table for completeness purposes.

The lowest chronic fish toxicity endpoint is a NOEC = 0.001 mg a.s./L, (nominal concentration) from a ELS study on *Oncorhynchus mykiss*. Based on this endpoint and taking into account that dimoxystrobin is not rapidly degradable (and has low potential for bioaccumulation), the DS proposes a classification of Aquatic Chronic 1 (H410) with an M factor of 100.

Comments received during consultation

Comments were received from four MSs. Three of the MSs explicitly supported the DS on the classification proposal, while requesting some clarifications on certain studies. The fourth member state requested clarification on the two key studies leading to classification, without commenting on the classification proposal. Clarifications were given by the DS in the RCOM document. The clarification/statement given by the DS regarding the key study by Anonymous (1999b), 1999/10521 (*Oncorhynchus mykiss*, NOEC = 0.001 mg a.s./L) lead to the conclusion by the DS that the study is invalid and should not be used for classification purposes. More explicitly the DS stated that the calculated hatching success in the control replicates as well as the mean hatching success were below the control survival validity criterion stipulated in the OECD TG 210.

Assessment and comparison with the classification criteria

Degradation

Dimoxystrobin is not considered by the DS to be readily biodegradable based on data from a valid study performed under OECD TG 301 F (Manometric Respirometry) (Werner, 1999). The degree of biodegradation (% biological oxygen demand/theoretical oxygen demand) over the 28-day test duration was in the range 0-10%, which is below the 60% of the theoretical

maximum criterion for readily degradable substances. This is also supported by limited mineralisation in an aerobic surface water-simulation biodegradation test (OECD TG 309) and long DT_{50}s in two water/sediment studies. Also, dimosystrobin was shown to be hydrolytically stable under high pH and temperatures. RAC agrees with the DS in concluding that dimoxystrobin is considered not rapidly degradable for the purpose of classification and labelling.

Bioaccumulation

Dimoxystrobin has a lipid-normalised kinetic bioconcentration factor BCF_{KL} = 91 L/kg which is below the criterion of BCF of ≥ 500 L/kg. It also has a log P_{OW} = 3.59, which is below the Log P_{OW} ≥ 4 criterion for substances with bioaccumulation potential. Thus, RAC agrees that dimoxystrobin is not bioaccumulative under CLP.

Aquatic Toxicity

The most sensitive species for acute aquatic toxicity is *Navicula pelliculosa* with an E_rC_{50} (72h) value of 0.0078 mg a.s./L, based measured concentrations. Acute toxicity for fish and invertebrates were reported for *Oncorhynchus mykiss* as LC_{50} (96h) = 0.0434 mg a.s./L (measured concentrations) and for *Daphnia magna* EC_{50} (48h) = 0.0394 mg a.s./L (nominal concentration). RAC supports the DS on the use of the *Navicula pelliculosa*, E_rC_{50} (72h) of 0.0078 mg a.s./L, based on measured concentrations, as the basis for the aquatic acute classification.

The most sensitive species for chronic aquatic toxicity is *Oncorhynchus mykiss* with a NOEC (97d) of 0.001 mg a.s./L, based on nominal concentrations. The lowest chronic toxicity endpoint for invertebrates was a *Daphnia magna* NOEC (21d) of 0.0125 mg a.s./L (nominal concentrations) and for algae an E_rC10 (72h) of 0.0035 mg a.s./L from the *Pseudokirchneriella subcapitata* study. The use of nominal concentration for the above-mentioned endpoints is valid as concentrations were maintained with in the acceptable range throughout the testing periods as stated in the respective guidelines. RAC considers the *Oncorhynchus mykiss* study as valid (more in-depth analysis is presented in a subsequent section) and agrees to use the derived NOEC value (=0.001 mg a.s./L) as the basis for the chronic classification.

Conclusion on classification

Based on an E_rC_{50} (72h) of 0.0078 mg a.s./L (*Navicula pelliculosa*), which is ≤1 mg/L, RAC agrees that dimoxystrobin warrants classification as **Aquatic Acute 1 (H400), M=100 (0.001 < L(E)C_{50} ⩽ 0.01 mg/L)**. Dimoxystrobin is not rapidly degradable and is not bioaccumulative in the aquatic environment. Based on the chronic endpoint NOEC (97d) of 0.001 mg a.s./L (*Oncorhynchus mykiss*), which is below 0.1 mg/L, RAC agrees that dimoxystrobin warrants classification as **Aquatic Chronic 1 (H410), M=100 (0.0001 < NOEC ⩽ 0.001 mg/L)**.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not relevant as no changes to the existing harmonized classification for dimoxystrobin are proposed.

12.1.1 Conclusion on classification and labelling for hazardous to the ozone layer

Dimoxystrobin is not classified as hazardous to the ozone layer.

13 ADDITIONAL LABELLING

Additional labelling requirements do not apply.

14 REFERENCES

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DIMOXYSTROBIN (ISO); (2*E*)-2-{2-[(2,5-DIMETHYLPHENOXY)METHYL]PHENYL}-2-(METHOXYIMINO)-*N*-METHYLACETAMIDE; (*E*)-2-(METHOXYIMINO)-*N*-METHYL-2-[α-(2,5-XYLYLOXY)-*o*-TOLYL]ACETAMIDE

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15 ANNEXES

Annex I is provided as a separate and confidential document.