Committee for Risk Assessment

RAC

Annex 1

Background document

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

trifloxystrobin (ISO); methyl (*E*)-methoxyimino-{(*E*)-α-[1-(α,α,α-trifluoro-*m*-tolyl)ethylideneaminooxy]-*o*-tolyl}acetate

EC Number: -

CAS Number: 141517-21-7

CLH-O-0000001412-86-293/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

20 September 2019

CLH report

Proposal for Harmonised Classification and Labelling

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

TRIFLOXYSTROBIN

EC Number: **Not assigned**

CAS Number: **141517-21-7**

Index Number: **607-424-00-0**

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Chemicals Regulation

Directorate

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United Kingdom

Version number: 2 **Date: November 2018**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON TRIFLOXYSTROBIN (ISO); METHYL (*E*)-METHOXYIMINO-{(*E*)-A-[1-(A,A,A-TRIFLUORO-*M*-TOLYL) ETHYLIDENEAMINOOXY] -*O*-TOLYL}ACETATE

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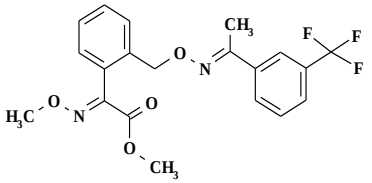
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON TRIFLOXYSTROBIN (ISO); METHYL (*E*)-METHOXYIMINO-{(*E*)-A-[1-(A,A,A-TRIFLUORO-*M*-TOLYL) ETHYLIDENEAMINOOXY] -*O*-TOLYL}ACETATE

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)	Trifloxystrobin (ISO); methyl (E)-methoxyimino-{(E)-α-[1-(α,α,α-trifluoro-m-tolyl)ethylideneaminooxy]-o-tolyl}acetate
Other names (usual name, trade name, abbreviation)	CGA 279202
ISO common name (if available and appropriate)	Trifloxystrobin (ISO accepted)
EC number (if available and appropriate)	not assigned
EC name (if available and appropriate)	not assigned
CAS number (if available)	141517-21-7
Other identity code (if available)	CGA 279202, AE C642802
Molecular formula	C20H19F3N2O4
Structural formula	
SMILES notation (if available)	C1C=C(C(=NOC)C(=O)OC)C(CON=C(C)C2C=CC=C(C(F)(F)F)C=2)=CC=1
Molecular weight or molecular weight range	408.38 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: 97.5%*

*The name shown above is that currently provided in Annex VI of CLP. The applicant has advised that the preferred name is methyl (2E)-(methoxyimino)(2-{[({(1E)-1-[3-(trifluoro-methyl)phenyl]ethylidene}amino)oxy]methyl}phenyl) acetate.

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)
Trifloxystrobin CGA 279202 CAS No.: 141517-21-7	97.5 – 99.7%	Skin Sens. 1, H317 Aquatic Acute 1, H400 Aquatic Chronic 1, H410	Skin Sens. 1, H317 Aquatic Acute 1, H400 Aquatic Chronic 1, H410

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

There are no impurities that are relevant for the classification.

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Not relevant	-	-	-	-	-

Table 5: Test substances (non-confidential information)

The test substance was considered to be the same as that outlined above. Details on the purity of the tested batches is provided in the following sections.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	607-424-00-0	trifloxystrobin (ISO); (E,E)-α-methoxyimino-{}{2-[[[[1-[3-(trifluoromethyl)phenyl]ethylidene]amino]oxy]methyl]benzeneacetic acid methyl ester	not assigned	141517-21-7	Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	GHS07 GHS09 Wng	H317 H410			
Dossier submitters proposal	607-424-00-0	trifloxystrobin (ISO); methyl (E)-methoxyimino-{(E)-α-[1-(α,α,α-trifluoro-m-tolyl)ethylideneaminooxy]-o-tolyl}acetate	not assigned	141517-21-7	**Retain** Aquatic Acute 1 **Retain** Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		**Add** M = 10 M = 10	
Resulting Annex VI entry if agreed by RAC and COM	607-424-00-0	trifloxystrobin (ISO); methyl (E)-methoxyimino-{(E)-α-[1-(α,α,α-trifluoro-m-tolyl)ethylideneaminooxy]-o-tolyl}acetate	not assigned	141517-21-7	Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	GHS07 GHS09 Wng	H317 H410		M = 10 M = 10	

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	hazard class not assessed in this dossier	No
Acute toxicity via dermal route	hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	hazard class not assessed in this dossier	No
Skin corrosion/irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	hazard class not assessed in this dossier	No
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	hazard class not assessed in this dossier	No
Germ cell mutagenicity	hazard class not assessed in this dossier	No
Carcinogenicity	hazard class not assessed in this dossier	No
Reproductive toxicity	**data conclusive but not sufficient for classification**	**Yes**
Specific target organ toxicity-single exposure	hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	hazard class not assessed in this dossier	No
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	**harmonised classification proposed**	**Yes**
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

To the applicant's knowledge, the hazard classification of trifloxystrobin according to the Dangerous Substances Directive 67/548/EEC (DSD) was first agreed in 2000 in meetings of the Commission Working Group on the Classification and Labelling of Dangerous Substances (Pesticides). The Working Group agreed to classify the substance with Xi; R43 and N; R50-53. The agreed classification was included in Annex I of the DSD, and later translated to the CLP Classification Skin Sens 1: H317, Aquatic Acute 1: H400 and Aquatic Chronic 1: H410 in Annex VI of Regulation (EC) No 1272/2008 (CLP Regulation).

RAC general comment

Trifloxystrobin is a pesticide active substance that controls diseases caused by plant pathogenic fungi across a wide range of agricultural and horticultural crops. During the renewal process, concerns for classification for reproductive toxicity were raised (albeit no new data were available), and M-factors were considered appropriate for the environmental classification. The CLH report therefore addresses only reproductive toxicity and hazardous to the aquatic environment.

Trifloxystrobin has an existing entry in Annex VI of CLP under the chemical name (*E*,*E*)-α-methoxyimino-{2-[[[[1- [3-(trifluoromethyl)phenyl]ethylidene]amino]oxy] methyl] benzeneacetic acid methyl ester. It is proposed to amend the name of the current Annex VI entry into methyl (*E*)-methoxyimino-{(*E*)-α-[1-(α,α,α-trifluoro-*m*-tolyl) ethylideneaminooxy]-*o*-tolyl}acetate, for consistency with the name used in the approval of the pesticide active substance and because the name includes a clerical error.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Trifloxystrobin is a pesticide active substance subject to renewal under Regulation (EC) 1107/2009, for which the UK is the Rapporteur Member State (RMS). The substance has an existing entry in Annex VI of CLP which includes classification for skin sensitisation and environmental hazards only. The available data on trifloxystrobin (as presented in this report) were considered previously at the Meeting of the Commission Working Group on the Classification and Labelling of Dangerous Substances, Pesticides – Health Effects (October 2000) and it was agreed that classification for human health was appropriate for skin sensitisation only. Whilst no new data are available, concerns for classification for reproductive toxicity were raised in the EFSA peer review process of the renewal during 2017, and a targeted review of the classification and labelling is considered appropriate to address these concerns. Regarding environmental classification M-factors are considered appropriate. This proposal therefore addresses only the following hazard classes: reproductive toxicity and hazardous to the aquatic environment. Data on repeated dose toxicity are also included in this dossier to assist the assessment of reproductive toxicity; however, the STOT RE end-point is not assessed.

5 IDENTIFIED USES

Trifloxystrobin provides protection to crops from the damage caused by plant pathogenic fungi. On the surface of the plant its primary biological mode of action is the inhibition of spore germination and germ tube extension, thereby preventing infection from taking place. In those fungi responsible for diseases such as powdery mildews which develop close to the outer layers of the host tissues after they have penetrated, control is also effected by the inhibition of the fungus within the plant tissues. The stages and processes

which are inhibited include mycelial growth, haustoria formation and sporulation. Trifloxystrobin is a contact fungicide with penetrant properties. The active substance is not translocated in the vascular system.

Trifloxystrobin controls diseases caused by pathogenic fungi from all four classes - *Ascomycetes, Deuteromycetes, Basidiomycetes* and *Oomycetes* across a wide range of agricultural and horticultural crops, including cereals, vines, soft fruit, top fruit, vegetables and ornamentals, grown in open field and/or under protection.

6 DATA SOURCES

Studies which have been submitted for Annex I renewal under 1107/2009.

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Test material (Batch no., purity)
Physical state at 20°C and 101,3 kPa	Active substance, pure: white, powder, odourless	Das, R.; 1996; M-041523-01-1	AMS 759/101: 99.7 %
	Active substance as manufactured: off-white powder, slightly sweet odour	Das, R.; 1997; M-041530-01-1	P.706029: 97.4 %
Melting/freezing point	Melting point: 72.9 °C	EU A1 Das, R.; 1996; M-041431-01-1	AMS 759/101: 99.7 %
Boiling point	approx. 312°C at 1013 hPa thermal decomposition starts at about 285°C	EU A2 Das, R.; 1996; M-041467-01-1	AMS 759/101: 99.7 %
Relative density	D_4^{20} = 1.36	EU A3 Fueldner, H.; 1997; M-041496-01-1	AMS 759/101: 99.7 %
Vapour pressure	$3.4 \cdot 10^{-6}$ Pa at 25 °C (extrapolated) from fit of measurements between 40 and 65 °C	EU A4 Widmer, H.; 1996; M-041511-01-1	AMS 759/101: 99.7 %
	Henry's law constant at 25 °C (calculated): $2.3 \cdot 10^{-3}$ Pa · m^3 · mol^{-1}	Burkhard, N.; 1997; M-041515-01-1	calculated
Surface tension	65.3-66.4 mN/m (filtrates of 0.1 g/L suspension) Trifloxystrobin is classified to be non-surface active.	OECD 115 Ryser, M.; 1997; M-043058-01-1	P.706029: 97.4 %
Water solubility	0.61 mg/L at 25 °C Because CGA 279202 has no dissociation constant in an accessible pH range, that means the pH has no effect to the water solubility of the compound in the pH range 4 to 10.	EU A6 Stulz, J.; 1997; M-041593-01-1	AMS 759/101: 99.7 %
Partition coefficient n-octanol/water	POW = 32000 ± (680) at 25 °C log POW = 4.5 ± (0.0094) at 25 °C	EU A8 Stulz, J.; 1997; M-041647-01-1	AMS 759/101: 99.7 %
Flash point	Not applicable (melting point > 40 °C).		

Property	Value	Reference	Test material (Batch no., purity)
Flammability	Not highly flammable in the sense of EC guideline A.10. On attempted ignition with a hot flame the substance melted. The molten substance did not sustain a flame.	EU A10 Angly, H.; 1997; M-041812-01-1	P.706029: 97.4 %
Explosive properties	The substance is not sensitive to thermal or mechanical (shock and friction) stimuli (EC guideline A.14)	EU A14 Angly, H.; 1997; M-041830-01-1	P.706029: 97.4 %
Thermal stability	No decomposition below 150 °C, only melting.	OECD 113 Angly, H.; 1997; M-041479-01-1	P.706029: 97.4 %
Self-ignition temperature	No self-ignition observed No significant observation on the temperature-time curve between room temperature and the melting point of the substance (approx. 70°C). EC guideline A.16.	EU A16 Angly, H.; 1997; M-041821-01-1	P.706029: 97.4 %
Oxidising properties	The maximum buring rate of the test substance mixture, 1.48 mm/s, was less than the buring rate of the reference mixture (barium nitrate), 3.6 mm/s). The substance was not considered an oxidizing substance (EC guideline A.17).	EU A17 Angly, H.; 1997; M-043079-01-1	P.706029: 97.4 %
Granulometry	No data		
Solubility in organic solvents	n-hexane 11 g/L at 25 °C 1-octanol 18 g/L at 25 °C methanol 76 g/L at 25 °C toluene 500 g/L at 25 °C ethyl acetate > 500 g/L at 25 °C acetone > 500 g/L at 25 °C dichloromethane > 500 g/L at 25 °C	CIPAC MT 157.3 Stulz, J.; 1997; M-041631-01-1 Stulz, J.; 1996; M-041643-01-1	P.706029: 97.4 %
Dissociation constant	Trifloxystrobin does not show any acidic or basic properties in the range of pH 2 and pH 12.	OECD 112 Stulz, J.; 1997; M-041749-01-1	AMS 759/101: 99.7 %
Viscosity	Not applicable.	–	–

8 EVALUATION OF PHYSICAL HAZARDS

Not addressed in this CLH report.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 9: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Similar to OECD TG 417. Balance (GP, TP)/tissue distribution (GP, TP)/tissue depletion (GP)/bile duct cannulation (GP) in male and female SD rats following single	The extent of absorption was dependent on dose level and sex. Peak blood concentration was reached 12-24 hours after dosing. At the low dose $AUC_{0\text{-last}}$ was independent of sex. The	Test substances: [glyoxyl-phenyl-U-^{14}C] trifloxystrobin (GP), [trifluoromethyl-phenyl-U-^{14}C]	Anonymous (1998a), M-136746-01-1

Method	Results	Remarks	Reference
oral dosing, balance and tissue distribution following repeat dosing. Dose levels: Single: 0.5 (GP) and 100 mg/kg bw (GP, TP) Repeat: 14 x 0.5 mg/kg bw/day (unlabelled) followed by 1 x 0.5 mg/kg bw (GP)	absorbed dose was rapidly eliminated with the majority of the dose eliminated in bile independent of sex, and there was some evidence of enterohepatic recirculation (apparent low recovery in high dose females GP). A sex difference was apparent in the amount of radioactivity excreted in urine (more in female urine). Tissue levels were low.	trifloxystrobin (TP) Vehicle: ethanol/PEG (3:5 v/v)	
Similar to OECD TG 417. Balance in male and female rats following single oral dosing at low dose (TP), tissue depletion in male and female rats following single low and high dose (TP), bile duct cannulation in female rats following single high dose (repeat of previous experiment GP). Dose levels: Single:0.5 and 100 mg/kg bw	Sex difference in route of elimination confirmed. Results of previous bile duct study confirmed, apparent low recovery attributed to miscalculation. Tissue depletion half life values generally 12-34 hours.	Test substances: [glyoxyl-phenyl-U-^{14}C] trifloxystrobin (GP), [trifluoromethyl-phenyl-U-^{14}C] trifloxystrobin (TP) Vehicle: ethanol/PEG (3:5 v/v)	Anonymous (1998b), M-136744-01-1
Similar to OECD TG 417. Samples from Anonymous 1998c and 1998b studies (M-136746-01-1 and M-136744-01-1) were processed to allow metabolite identification by MS ^{1}H- and/or ^{13}C- and NMR spectroscopy LC-MS and HPLC / 2D-TLC co-chromatography with reference standards.	Major metabolic pathways were hydrolysis of the methyl ester, demethylation of the methoxy imino group, oxidation of the methyl side chain and cleavage between the glyoxyl phenyl and trifluoromethylphenyl moiety. Major pathways were influenced by sex since oxidation of methyl side chain was more pronounced in females.	-	Anonymous (1998c), M-136745-01-1; Anonymous (1998b), M-136744-01-1
Comparative *in vitro* metabolism study – no TG available. Test substance (15 µM) was incubated for one hour with liver microsomes from male Wistar rats and humans of both genders (protein conc. 1 mg/mL) at 37°C. Radioactive components from each incubation were separated by radio-HPLC. Positive control incubation was conducted.	Radioactive recovery in microsome incubations amounted to 91 and 96% of applied radioactivity in rat and human liver microsomes, respectively. Metabolic activity of microsomes was demonstrated with positive control. Rats appeared to generate more metabolites than humans. No human specific metabolites were generated.	Test substance: [trifluoromethyl-phenyl-U-^{14}C] trifloxystrobin (TP)	Solà, J.; 2015; M-473161-02-1

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Absorption

Trifloxystrobin was moderately absorbed via the intestinal tract. 56 and 65% of the administered dose were absorbed at the low dose level (0.5 mg/kg bw) by male and female rats after 48 h, respectively, as demonstrated by bile-duct cannulated rats. At the high dose level (100 mg/kg bw) absorption decreased to 55 and 45% of the

administered dose in male and female rats, respectively. The rate and extent of absorption of the total radioactivity was essentially independent of sex and the labelling position.

Maximum residues in blood were reached between 12 and 24 hours after dosing independent of the dose level, sex of the animal and site of label (GP and TP radiolabel). At low dose (0.5 mg/kg bw), the areas under the curve ($AUC_{0\text{-}96h}$) were in the same range for females and males indicating an equal bioavailability. At the high dose, the AUC value increased to only 130-times compared with a dose level of 200:1 as a result from the lower absorption at the high dose level.

Distribution

Residues in tissues were widely distributed. At low dose, maximum residues in tissues were reached at the time of maximal blood residues irrespective of the sex of the animals. The residual radioactivity depleted from tissues and organs with half life times of 14 to 40 hours except of blood ($t_{½}$ = 30-82 h) and spleen ($t_{½}$ = 38-68 h). Highest residues in tissues and depletion from tissues were independent from dose level and sex of animals. The sum of total residues in organs and tissues amounted to ≤ 0.5% of the administered dose 7 days after administration. There was no evidence of accumulation.

At high dose and 7 days after administration, highest residues were found in the organs responsible for the distribution, degradation, and excretion, i.e. blood, kidneys and liver. Residues in all tissues were higher in females than in males except of liver ([glyoxyl-phenyl-U-^{14}C] label) and plasma (both labels).

Metabolism

Trifloxystrobin was extensively metabolised in the rat. 35 metabolites were found in urine, faeces and bile. The amount of identified metabolites ranged between 60 and 70% of the administered dose. Major pathways included the 1) hydrolysis of the methyl ester to the corresponding acid, 2) O-demethylation of the methoxyimino group to the hydroxyimino compound, 3) the oxidation of the methyl side chain to a primary alcohol and partial oxidation to the respective carboxylic acid and 4) cleavage between the glyoxyl phenyl and trifluoromethyl phenyl rings. Major metabolic pathways of trifloxystrobin were not dependent on the dose or pretreatment, but on the sex of the animals resulting in female specific urinary metabolites. Bile metabolites were mostly glucuronic and tentatively sulphuric acid conjugates. The extent of metabolism was dependent on dose and absorption. At the low dose level degradation was almost complete whereas at the high dose level 31-47% was excreted unchanged with the faeces.

The comparative metabolism of [trifluoromethylphenyl-UL-^{14}C]trifloxystrobin was investigated in *in-vitro* systems by incubating the test substance (15 µM) with liver microsomes (protein concentration 1 mg/mL) from male rats and humans of both genders in the presence of NADPH cofactor for one hour at 37°C. All metabolites formed by humans were also formed by rats. Human liver microsomes do not generate any unique metabolite that was not formed by rat liver microsomes.

Elimination

The majority of radioactivity was eliminated within 48 hours: 72 – 96% of the administered dose was excreted with urine, faeces and bile after 48 hours. The extent of excretion was independent of the dose level, pretreatment with unlabelled trifloxystrobin or sex of the animals. Seven days after administration the dose was almost completely eliminated. Female rats eliminated twice the amount of the radioactivity in the urine than males. However, the major route of elimination was via bile in both sexes (ca. 44% of the dose). At the low dose, more than half of the dose excreted with the faeces was derived from biliary excretion. There was evidence of the involvement of enterohepatic circulation in the excretion process.

The sex dependent excretion pattern and sex-related differences in tissue residues indicated quantitative and/or qualitative differences in the metabolism of trifloxystrobin in male and female rats. This gender based difference in elimination route was considered to be a result of unique metabolism in females and not related to differences in relative abundance and preferred route of elimination of common metabolites.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Not considered in this report.

10.2 Acute toxicity - dermal route

Not considered in this report.

10.3 Acute toxicity - inhalation route

Not considered in this report.

10.4 Skin corrosion/irritation

Not considered in this report.

10.5 Serious eye damage/eye irritation

Not considered in this report.

10.6 Respiratory sensitisation

Not considered in this report.

10.7 Skin sensitisation

Not considered in this report.

10.8 Germ cell mutagenicity

Not considered in this report.

10.9 Carcinogenicity

Not considered in this report.

10.10 Reproductive toxicity

The reproductive toxicity of trifloxystrobin has been investigated in a two-generation study in rats and developmental toxicity studies in rats and rabbits. The results of these studies are summarised in Table 10, 11 and 12. Further information about the studies is provided below the tables, and in Annex I.

10.10.1 Adverse effects on sexual function and fertility

A two-generation study in rats is available to investigate the effects of trifloxystrobin on sexual function and fertility.

Table 10: Summary table of animal studies on adverse effects on sexual function and fertility

Study, species (strain)	Dose levels	Key Observations
Range-finding reproductive study Oral (dietary administration) Non guideline Non GLP Rats, Tif RAIf (SPF) 15/sex/group Trifloxystrobin (purity 96.4 %) Anonymous (1995), M-053434-01-1	0, 100, 1000, 2000 ppm Males: 0, 6.0, 53.5, 109.6 mg/kg bw/d Females: 0, 7.9 – 16.5, 67.0 – 168.6, 140.5 – 321.71 mg/kg bw/d Treatment started 2 weeks before mating and continued throughout gestation and lactation until post partum day 14	100 ppm No treatment related effects 1000 ppm Decreased food consumption (by up to 14%) Decreased parental body weight (by up to 6%) 2000 ppm Decreased food consumption (by up to 15%) Decreased parental body weight (by up to 8%) Decreased pup body weight from LD7 onwards (by up to 19%)
Two-generation study in rats Oral (dietary administration) OECD 416 GLP Rats, Tif RAIf (SPF) 30/sex/group Trifloxystrobin (purity 96.4 %) Anonymous (2001), M-039264-02-1	0, 50, 750, 1500 ppm Males: 0, 2.3 – 3.8, 32.9 – 58.4, 73.1 – 126.7 mg/kg bw/d Females: 0, 3.1 – 8.0, 47.9 – 119.9, 98.0 – 242.0 mg/kg bw/d	**Parental toxicity** **F0 and F1 generation** 50 ppm No effects 750 ppm (males and females): Decreased food consumption (by up to 15 % in F0 animals; up to 20 % in F1 animals) and body weight (by up to 8 % in F0 animals; up to 31 % in F1 animals) Increased incidence of animals with minimal to moderate hypertrophy of centrilobular hepatocytes, and males (F0) with minimal pigmentation of renal tubules 1500 ppm (males and females): Decreased food consumption (by up to 16 % in F0 animals; up to 23 % in F1 animals) and body weight (by up to 13 % in F0 animals; up to 30 % in F1 animals) Increased incidence of animals with minimal to moderate hypertrophy of centrilobular hepatocytes, and incidence of F0 animals with minimal pigmentation of renal tubules
		Fertility / Reproduction **F0 and F1 generation** No treatment-related effects on reproductive parameters up to the highest dose tested reproductive NOAEL: 73 mg/kg bw/day (EFSA conclusion on pesticides peer review, 2017-09-14)
		Offspring toxicity

Study, species (strain)	Dose levels	Key Observations
		F1 and F2 pups 50 ppm No treatment-related effects 750 ppm (males and females): Decreased body weight from LD14 (LD7 in F2 pups) onwards (by up to 11 % in F1 pups; up to 14 % in F2 pups) 1500 ppm (males and females): Decreased body weight from LD4 onwards (by up to 27 % in F1 pups; up to 28 % in F2 pups) Retarded achievement of physical/ behavioral landmarks (eye opening) in F1 and F2 pups offspring NOAEL: 2.3 mg/kg bw/day (EFSA conclusion on pesticides peer review, 2017-09-14)

LD = lactation day

In a preliminary range finding generation study, trifloxystrobin was administered via the diet to groups of 15 rats/sex/dose level at dietary concentrations of 0, 100, 1000 or 2000 ppm. In this study parental toxicity was evident at the mid and high doses by reduced food intake and reduced body weight and body weight gain. Mortality, clinical signs or remarkable observations at gross necropsy were not observed. Reproduction was not affected at any dose. At 2000 ppm, body weight development of the offspring was decreased from lactation day 7 onwards. Based on these findings an appropriate high dose level of 1500 ppm for a two-generation study with trifloxystrobin was recommended.

In a guideline two-generation study in rats, trifloxystrobin was administered *via* the diet to groups of 30 rats/sex/dose level at fixed dietary concentrations of 0, 50, 750 or 1500 ppm.

After 10 weeks premating dietary exposure to the test substance, animals were paired 1:1 within each dose group (30 animals per sex and dose) until there was evidence of positive mating or for 19 days, whichever occurred first. Litters were culled to 4 male and 4 female pups, where possible, on day 4 post partum. After weaning of the F1a pups, the F0 parent animals were re-mated to produce second litters (F1b pups). The F1 generation was selected from the first litters (i.e. F1a pups) of the F0 generation. The same group sizes and doses were repeated to produce the F2 generation.

Clinical signs, bodyweights, food consumption, mating, gestation and delivery parameters, pup survival and development were recorded. A gross necropsy examination was performed on all pups not selected for mating. Parent animals were necropsied after weaning of the second (F0 parents) or first (F1 parents) litters and subjected to macroscopic examination, with histopathological investigation (in all control and high dose animals) of vagina, uterus, ovaries, testes, epididymides, seminal vesicles, prostate, pituitary gland, liver, pancreas and all gross lesions.

Parental toxicity

F0 Generation

There were no treatment-related clinical signs or treatment-related deaths among the F0 parent animals.

At 1500 ppm, body weights were significantly lower than controls in males throughout the F0 generation (by up to 12%) and in females during the pre-mating period (by up to 8%). Body weight gain was also reduced during these periods (by up to 24% in males, and 17% in females).

During the two gestation periods, overall body weight gain was lower in females at ≥750 ppm, leading to reduced body weights in these animals (by up to 12% at the top dose).

During the two lactation periods, overall weight gain was increased in females at ≥750 ppm (by 355% at 1500 ppm). However, bodyweights during the two lactation periods remained lower than controls at ≥ 750 ppm; up to 9% and 13% lower in lactation periods 1 and 2, respectively.

The effects on body weight and body weight gain were generally consistent with the effects seen on food consumption. During the premating period food consumption was slightly reduced (<10% lower than controls) from the start of the dosing period at 1500 ppm (both sexes), and 750 ppm (males only). For females, during the premating period, food consumption was generally slightly lower (1st week - 15%, up to -10% thereafter) than that of the control group.

For the females at ≥ 750 ppm, there was a reduction in food consumption by up to 15% compared with controls during the gestation and the lactation periods (second mate) and during days 14 and 21 post partum of the first lactation period.

There were no treatment related macroscopic findings during necropsy of the F0 generation at termination. At 1500 ppm, absolute spleen weights (males), adrenal and brain weights (female) were significantly reduced. For both sexes, relative weights of most organs were increased and significantly different from the respective control value. At 750 ppm, absolute brain weights were slightly but significantly reduced; and relative liver and ovaries weights were slightly but significantly increased in females. In males, relative kidneys and liver weights were minimally but significantly increased.

The changes in absolute and relative organ weights were attributed primarily to the reduced body weights of treated animals compared with controls, and were thought not to be a specific toxic effect on target organs. For example, absolute brain weights were 3-6% lower than controls but relative weights were 8-11% higher than controls at 1500 ppm. Other relative organ weights in treated groups were between 8-16% higher than controls which is consistent with terminal bodyweights that were 11-13% lower than controls.

No treatment-related changes were observed at histopathological examination of the reproductive organs of the control and high dose (1500 ppm) groups.

Microscopic examination of the liver showed an increased incidence of males and females at 1500 ppm with minimal hypertrophy of centrilobular hepatocytes. Microscopic examination of the kidneys showed an increased incidence of males and females at 1500 ppm and of males at 750 ppm with minimal pigmentation of renal tubules. A decreased incidence of males and females with splenic hemosiderosis was noted at ≥ 750 ppm.

<u>F1 Generation</u>

There were no treatment-related clinical signs or treatment-related deaths among the F1 parent animals.

The selected F1 animals were representative of the F1a generation in that the 750 ppm and 1500 ppm dose groups had lower bodyweights (by up to 11% at 750 ppm and up to 30% at 1500 ppm). Throughout the F1 generation, bodyweights in both groups (both sexes) remained significantly lower than controls, but bodyweight gain was usually similar to that of the control group.

During the gestation period, overall weight gain of the females was significantly lower than controls at ≥ 750 ppm (by up to 9% at 750 ppm, and up to 24% at 1500 ppm). The resulting lower bodyweights during the gestation period were significantly different from controls on all occasions. This effect was more pronounced in the high dose group than in the mid dose group (↓8% at 750 ppm, ↓17% at 1500 ppm).

As in the F0 generation, for the females at ≥ 750 ppm, overall weight gain during the lactation period was superior to that of the control group (by 299% at 1500 ppm).

At 1500 ppm (both sexes) and at 750 (females), food consumption was reduced and usually significantly different from the control group throughout the F1 generation, including during the lactation periods (typically less than 10% but sometimes up to 15% lower than controls).

As with the F0 generation there were no treatment related macroscopic findings during necropsy of the F1 generation parents. At 1500 ppm, male spleen and brain weights and female brain and kidneys weights were the only absolute organ weights which were significantly lower than the respective control value. At 750 ppm, male brain weights and female kidneys and liver weights were significantly lower than the controls. At 1500 ppm, (for both sexes) and at 750 ppm (females), relative organ weights of most organs were increased and significantly different from the respective control value. These organ weight changes were considered to be due to the decreased bodyweights and not to be a specific toxic effect on target organs. The pattern of organ weight differences between treated groups and controls was consistent with that observed for the F0 parents. Relative organ weights were around 11 to 19% higher than controls but this reflected a greater effect on F1 terminal body weights (15-17% lower than controls) compared with the F0 generation.

Microscopic examination of the liver showed an increased incidence of males and females at ≥ 750 ppm with minimal to moderate hypertrophy of centrilobular hepatocytes. Microscopic examination of the spleen showed decreased incidence of males and females with splenic hemosiderosis at ≥ 750 ppm.

Male reproductive parameters

There were no treatment related effects on male mating or fertility indices in either generation.

Female reproductive parameters

There were no treatment-related effects on mating and fertility indices, maternal gestation and parturition indices and the duration of gestation were unaffected by treatment at either mating in either the F0 or the F1 females.

Offspring toxicity

See Section 10.10.4 – Adverse effects on development

Conclusion

In the 2 generation toxicity study in rats, there were no effects on reproductive parameters. At microscopic examination, no treatment-related changes were observed in the reproductive organs. This is consistent with the results of 90 day and 2 year toxicity studies (Section 10.12).

Parental toxicity was observed in the top and mid-dose groups (750 and 1500 ppm), and comprised reduced food consumption, body weight and body weight gain compared to controls in both sexes of each generation. The effect was greater in the F1 than the F0 animals. However, during the lactation periods, body weight gain of the females (F0 and F1) was increased at 750 and 1500 ppm, despite food consumption being lower than in controls. Overall body weights remained lower than controls in these animals.

Other treatment-related parental findings were an increased incidence of minimal to moderate hypertrophy of centrilobular hepatocytes in males and females (both generations at 1500 ppm, and F1 generation at 750 ppm). There was also an increased incidence of F0 animals with minimal pigmentation of renal tubules in the 1500 ppm group and in F0 males in the 750 ppm group.

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

The potential of trifloxystrobin to adversely affect fertility, pregnancy outcome, and post-natal offspring survival and development has been investigated in a guideline two-generation reproduction study in Tif RAIF rats.

Parental toxicity was evident in groups receiving doses ≥ 750 ppm as reductions in food consumption and body weights, with more marked effects seen at the top dose (1500 ppm). There was also evidence of slight toxicity to the liver and kidneys of parental animals at these dose levels.

There were no treatment related effects on fertility and mating indices, or on female reproductive indices.

Overall, the dossier submitter concludes that a specific effect on fertility, reproduction and pregnancy outcome was not demonstrated by this study.

10.10.3 Comparison with the CLP criteria

In the available study on trifloxystrobin, there was no evidence of effects on fertility or reproductive performance. Therefore, the substance does not meet the criteria for classification.

Not classified. Data conclusive but not sufficient for classification.

10.10.4 Adverse effects on development

The developmental toxicity of trifloxystrobin has been investigated in guideline studies in rats and rabbits. These studies are summarised in Table 12. In addition, information on developmental toxicity is available from the 2 generation study discussed in Section 10.10.1. Effects from this study that are relevant for the assessment of developmental toxicity are discussed below, and in Table 11.

2 Generation Study (see summary in Table 10)

F1a Pups

At birth, mean litter size did not differ between groups. The sex ratios on days 0 and 21 post partum were similar in all groups. Both the viability index (percentage of pups surviving days 0 to 4 post partum) and the lactation index (percentage of pups surviving days 4 to 21 post partum) were comparable in all groups. In the control, 50, 750 and 1500 ppm dose groups, respectively, 30, 29, 29 and 27 dams successfully reared their litters to weaning on day 21 post partum.

Mean pup weight at birth was similar in all groups. At 1500 ppm, there was a marked reduction in weight gain of pups throughout the lactation period with the result that mean pup bodyweights were significantly lower than controls from day 7 post partum through to weaning on day 21 postpartum (mean body weight was 28% lower in both sexes on day 21, compared to controls). At 750 ppm, there was a retardation of pup weight gain, such that mean pup bodyweights were significantly lower than controls on days 14 and 21 post partum (mean body weight was 9% lower in both sexes on day 21, compared to controls). At 50 ppm, mean pup weights and mean pup weight gain were similar to that of the control group after culling on day 4 post partum through to weaning on day 21 post partum.

For the pups at 1500 ppm, mean values for eye opening were delayed by 0.7 days in comparison to the control group. Differences from the control value were statistically significant. No treatment-related macroscopic findings were noted at necropsy of the F1a pups not chosen as F1 parents.

F1b Pups

At birth, mean litter size did not differ between groups. The sex ratios on days 0 and 21 post partum were similar in all groups. Both the viability index and the lactation index were comparable in all groups. In the control, 50, 750 and 1500 ppm dose groups, respectively, 28, 25, 29 and 28 dams successfully reared their litters to weaning on day 21 post partum.

Mean pup weight at birth was similar in all groups. At 1500 ppm, there was a marked reduction in weight gain of pups throughout the lactation period with the result that mean pup bodyweights were significantly lower than controls from day 7 post partum through to weaning on day 21 postpartum (mean body weight was 27% lower in both sexes on day 21, compared to controls). At 750 ppm, there was a retardation of pup weight gain after culling on day 4 post partum, such that mean pup bodyweights were significantly lower than controls on days 14 and 21 post partum (mean body weight was 12/11% lower than controls in males and females

respectively). At 50 ppm mean pup weights and mean pup weight gain were similar to that of the control group after culling on day 4 post partum through to weaning on day 21 post partum.

For the pups at 1500 ppm, mean values for eye opening were significantly delayed by 0.6 days in comparison to the control group. No treatment-related macroscopic findings were noted at necropsy of the F1b pups.

F2 Pups

At birth, mean litter size did not differ between groups. The sex ratios on days 0 and 21 post partum were similar in all groups. Both the viability index and the lactation index were comparable in all groups. In the control, 50, 750 and 1500 ppm dose groups, respectively, 28, 28, 28 and 29 dams successfully reared their litters to weaning on day 21 post partum.

Mean pup weight at birth was similar in all groups. There was a retardation in weight gain at 750 and 1500 ppm throughout the lactation period, such that mean bodyweights in both sexes were significantly lower than controls (~18% lower by day 21 at 750 ppm; ~29% lower by day 21 at 1500 ppm)). At 50 ppm, mean pup weights and mean pup weight gain were similar to that of the control group after culling on day 4 post partum, through to weaning on day 21 post partum.

For the pups at 1500 ppm, mean values for eye opening were significantly delayed by 0.7 days in comparison to the control group. No treatment-related macroscopic findings were noted at necropsy of the F2 pups.

Table 11: Table showing pup weights during lactaction (F1a, F1b and F2 pups)

Parameter	**Generation**	**Dose (ppm)**						
		0	**50**	*(%)[a]*	**750**	*(%)[a]*	**1500**	*(%)[a]*
Males & females combined								
LD 0 birth	F1a	6.1	6.1	*(100%)*	6.1	*(100%)*	6.2	*(102%)*
LD 4 prior reduction		9.2	9.4	*(102%)*	9.3	*(101%)*	8.6	*(93%)*
LD 4 after reduction		9.2	9.5	*(103%)*	9.3	*(101%)*	8.6	*(93%)*
LD 7 (week 1)		15	15.2	*(101%)*	14.5	*(97%)*	**12.7****	**(85%)**
LD 14 (week 2)		30.1	30.2	*(100%)*	**28.3***	***(94%)***	**23.7****	**(79%)**
LD 21 (week 3)		49.3	49	*(99%)*	**44.7****	***(91%)***	**35.3****	**(72%)**
Males								
LD 0 birth	F1a	6.2	6.3	*(102%)*	6.3	*(102%)*	6.4	*(103%)*
LD 4 prior reduction		9.4	9.7	*(103%)*	9.5	*(101%)*	8.8	*(94%)*
LD 4 after reduction		9.4	9.7	*(103%)*	9.5	*(101%)*	8.8	*(94%)*
LD 7 (week 1)		15.3	15.6	*(102%)*	15	*(98%)*	**12.9****	***(84%)***
LD 14 (week 2)		30.7	30.8	*(100%)*	**29***	***(94%)***	**24.2****	***(79%)***
LD 21 (week 3)		50.3	50.4	*(100%)*	**46***	***(91%)***	**36.1****	***(72%)***
Females								
LD 0 birth	F1a	5.9	5.9	*(100%)*	6	*(102%)*	6.0	*(102%)*
LD 4 prior reduction		8.9	9.1	*(102%)*	9	*(101%)*	8.3	*(93%)*
LD 4 after reduction		9	9.3	*(103%)*	9.1	*(101%)*	8.3	*(92%)*
LD 7 (week 1)		14.7	15	*(102%)*	14.2	*(97%)*	**12.3****	***(84%)***
LD 14 (week 2)		29.5	29.8	*(101%)*	**27.8***	***(94%)***	**23.2****	***(79%)***
LD 21 (week 3)		48.3	47.7	*(99%)*	**43.9***	***(91%)***	**35****	***(72%)***
Males & females combined								
LD 0 birth	F1b	5.9	6.2	*(105%)*	6	*(102%)*	6.3	*(107%)*
LD 4 prior reduction		8.7	9.1	*(105%)*	8.7	*(100%)*	8.2	*(94%)*
LD 4 after reduction		8.9	9.3	*(104%)*	8.8	*(99%)*	8.3	*(93%)*
LD 7 (week 1)		14.5	15	*(103%)*	13.7	*(94%)*	**12.2****	***(84%)***
LD 14 (week 2)		30.2	30.6	*(101%)*	**27.4****	***(91%)***	**23.6****	***(78%)***
LD 21 (week 3)		51.8	52	*(100%)*	**46.2****	***(89%)***	**37.7****	***(73%)***
Males								
LD 0 birth	F1b	6.1	6.3	*(103%)*	6.3	*(103%)*	6.4	*(105%)*
LD 4 prior reduction		8.9	9.3	*(104%)*	8.9	*(100%)*	8.4	*(94%)*
LD 4 after reduction		9.1	9.5	*(104%)*	9.1	*(100%)*	8.5	*(93%)*
LD 7 (week 1)		14.7	15.4	*(105%)*	13.9	*(95%)*	**12.5****	***(85%)***
LD 14 (week 2)		30.6	31.3	*(102%)*	**27.7****	***(91%)***	**24.0****	***(78%)***
LD 21 (week 3)		53.1	53.6	*(101%)*	**46.9****	***(88%)***	**38.6****	***(73%)***
Females								
LD 0 birth	F1b	5.7	6.0	*(105%)*	5.8	*(102%)*	6.0	*(105%)*
LD 4 prior reduction		8.5	8.9	*(105%)*	8.4	*(99%)*	8.0	*(94%)*
LD 4 after reduction		8.7	9.1	*(105%)*	8.5	*(98%)*	8.1	*(93%)*
LD 7 (week 1)		14.2	14.7	*(104%)*	13.3	*(94%)*	**12.0****	***(85%)***
LD 14 (week 2)		29.8	29.8	*(100%)*	**27.0****	***(91%)***	**23.1****	***(78%)***
LD 21 (week 3)		50.6	50.2	*(99%)*	**45.2****	***(89%)***	**36.7****	***(73%)***
Males & females combined								
LD 0 birth	F2	6.0	6.1	*(102%)*	6.0	*(100%)*	6.1	*(102%)*
LD 4 prior reduction		9.0	9.3	*(103%)*	8.7	*(97%)*	8.5	*(94%)*
LD 4 after reduction		9.1	9.4	*(103%)*	8.8	*(97%)*	8.5	*(93%)*
LD 7 (week 1)		14.9	15.0	*(101%)*	**13.6****	***(91%)***	**12.5****	***(84%)***
LD 14 (week 2)		29.8	30.2	*(101%)*	**26.6****	***(89%)***	**23.3****	***(78%)***
LD 21 (week 3)		51.8	51.8	*(100%)*	**44.5****	***(86%)***	**37.3****	***(72%)***
Males								
LD 0 birth	F2	6.3	6.3	*(100%)*	6.2	*(98%)*	6.3	*(100%)*
LD 4 prior reduction		9.2	9.5	*(103%)*	8.8	*(96%)*	8.6	*(93%)*

Parameter	**Generation**	**Dose (ppm)**					
		0	**50**	*(%)[a]*	**750**	*(%)[a]*	**1500**	*(%)[a]*
LD 4 after reduction		9.3	9.5	*(102%)*	8.9	*(96%)*	8.7	*(94%)*
LD 7 (week 1)		15.1	15.2	*(101%)*	**13.6****	***(90%)***	**12.7****	***(84%)***
LD 14 (week 2)		30.1	30.7	*(102%)*	**26.4****	***(88%)***	**23.7****	***(79%)***
LD 21 (week 3)		53.3	53.1	*(100%)*	**44.4****	***(83%)***	**38.1****	***(71%)***
Females								
LD 0 birth	F2	5.8	5.9	*(102%)*	5.8	*(100%)*	5.9	*(102%)*
LD 4 prior reduction		9.0	9.1	*(101%)*	8.5	*(94%)*	8.3	*(92%)*
LD 4 after reduction		9.1	9.3	*(102%)*	8.6	*(95%)*	**8.3***	***(91%)***
LD 7 (week 1)		14.7	14.8	*(101%)*	**13.5***	***(92%)***	**12.2****	***(83%)***
LD 14 (week 2)		29.3	29.8	*(102%)*	**26.6****	***(91%)***	**22.9****	***(78%)***
LD 21 (week 3)		50.4	50.5	*(100%)*	**44.2****	***(88%)***	**36.5****	***(72%)***

[a] % of control

LD: lactation day* statistically significant difference from control $p<0.05$, ** statistically significant difference from control $p<0.01$

Table 12: Summary table of animal studies on adverse effects on development

See next page.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON TRIFLOXYSTROBIN (ISO); METHYL (*E*)-METHOXYIMINO-{(*E*)-A-[1-(A,A,A-TRIFLUORO-*M*-TOLYL) ETHYLIDENEAMINOOXY] -*O*-TOLYL}ACETATE

<table>
<tr><th>Study, species (strain)</th><th>Dose levels</th><th>Critical effects</th></tr>
<tr><td>Developmental range-finding study
Oral (gavage)
Non guideline
Non GLP
Rats, Tif RAIf (SPF)
Females
7/group
Trifloxystrobin (purity not specified)
Vehicle: 0.5 % w/w carboxy-methylcellulose
Anonymous (1993), M-052919-01-1</td><td>0, 10, 100, 1000 mg/kg bw/d
from gestation day 6-15</td><td><u>There were no deaths or treatment related clinical signs at any dose</u>

<u>10 mg/kg bw/d:</u>
<u>None</u>

<u>100 mg/kg bw/d:</u>
<u>None</u>

<u>1000 mg/kg bw/d:</u>
Decreased food consumption (by up to 12% during treatment period), decreased body weight gain (by up to 18% during treatment period)</td></tr>
<tr><td>Developmental toxicity study in rats
Oral (gavage)
OECD 414
GLP
Rats, Tif:RAIf (SPF)
Females
24/group
Trifloxystrobin (purity 96.4 %)
Vehicle: 0.5 % w/w carboxy-methylcellulose
Anonymous (1999a), M-039420-02-1</td><td>0, 10, 100, 1000 mg/kg bw/d
from gestation day 6-15</td><td><u>Maternal toxicity</u>
<u>100 mg/kg bw/d:</u>
Decreased food consumption (by 8% during treatment period) and body weight gain (18% by day 21)

<u>1000 mg/kg bw/d:</u>
Decreased food consumption (by 30% during treatment period), body weight gain (32% by day 21) and body weight (by 6% during treatment period)

maternal NOAEL: 10 mg/kg bw/day (EFSA conclusion on pesticides peer review, 2017-09-14)

<u>Developmental toxicity</u>
<u>1000 mg/kg bw/d:</u>
Statistically significant increase in the incidence of enlarged fetal thymus (11 out of 146)

Foetal (litter) incidences (%) of enlarged fetal thymus
<table>
<tr><td>Dose levels (mg/kg bw/d)</td><td>0</td><td>10</td><td>100</td><td>1000</td><td>HCD range[a]</td></tr>
<tr><td>Enlarged thymus</td><td>2.0 (13.0)</td><td>2.2 (4.5)</td><td>2.2 (15.0)</td><td>7.5* (31.8)</td><td>0-6.0 (0-29.2)</td></tr>
</table>
* Statistically significant ($p<0.05$)
[a] Data 22 studies, 4793 fetuses, 725 litters, conducted 1988-1994, same laboratory and strain

developmental NOAEL: 100 mg/kg bw/day (EFSA conclusion on pesticides peer review, 2017-09-14)</td></tr>
</table>

Developmental range-finding study Oral (gavage) Non guideline Non GLP Rabbits, Russian Chbb:HM Females 5/group Trifloxystrobin (purity 97.1 %) Vehicle: 0.5 % w/w carboxy-methylcellulose Anonymous (1994a), M-053339-01-1	0, 20, 100, 500, 1000 mg/kg bw/d from gestation day 7-19	There were no deaths at any dose. 20 mg/kg bw/d: None 100 mg/kg bw/d: Decreased food consumption (by up to 57% during treatment), decreased body weight and body weight gain (by up to 104% during treatment period), lower gravid uterus weight (by up to 17%) 500 mg/kg bw/d: Decreased food consumption (by up to 77% during treatment), decreased body weight (by 12% at end of treatment) and body weight gain (by up to 241% during treatment period), lower gravid uterus weight (by up to 20%), increase of postimplantation losses (one dam with total loss of implant), decreased litter size, reduced foetal weight 1000 mg/kg bw/d: Food consumption and body weight not reported, reduced activity, haemorrhagic discharge, no viable fetuses
Developmental toxicity study in rabbits Oral (gavage) OECD 414 GLP Rabbits, Russian Chbb:HM Females 19/group Trifloxystrobin (purity 96.4 %) Vehicle: 0.5 % w/w carboxy-methylcellulose Anonymous (1999b), M-000780-01-1	0, 10, 50, 250, 500 mg/kg bw/d from gestation day 7-19	**Maternal toxicity** 250 mg/kg bw/d: Decreased food consumption (by > 50%), body weight gain (↓130%) and body weight (↓6%) during treatment period 500 mg/kg bw/d: Decreased food consumption (by >50%), body weight gain (↓238%) and body weight (by up to 8%) during treatment period maternal NOAEL: 50 mg/kg bw/day (EFSA conclusion on pesticides peer review, 2017-09-14) **Developmental toxicity** 250 mg/kg bw/d: Increased incidence of asymmetric and/or fused/partially fused sternebrae (see Tables 15, 16 and 17) 500 mg/kg bw/d: Increased incidence of asymmetric and/or fused/partially fused sternebrae (see Tabe 15, 16 and 17) developmental NOAEL: 50 mg/kg bw/day (EFSA conclusion on pesticides peer review, 2017-09-14)

10.10.4.1 Developmental toxicity in rats

In a preliminary range finding developmental study, trifloxystrobin was administered to four groups of seven mated Sprague Dawley derived rats by gavage daily from day 6 through 15 of gestation at dose levels of 0, 10, 100 and 1000 mg/kg bw/day. There were no deaths or treatment-related clinical signs at any dose. Food consumption and body weight gain was slightly reduced in the highest dose group of 1000 mg/kg bw/day during the treatment period. Thus, the same dose levels were considered appropriate for the conduct of the main study.

Trifloxystrobin (purity 96.4%) was administered orally by gavage to mated female Sprague Dawley derived rats (20-23/dose) dissolved in 0.5% aqueous Na-carboxymethylcellulose at levels of 0, 10, 100 and 1000 mg/kg bw/day from gestation day 6 through gestation day 15. Animals were observed daily for clinical signs of toxicity. Bodyweights and food consumption were measured regularly. All females were sacrificed on gestation day 20 and subjected to a gross necropsy and caesarean section. Fetuses were individually weighed, sexed, and examined for external, skeletal and visceral abnormalities.

All dams survived until terminal sacrifice. Haemorrhagic discharge in the perineal region was seen in one dam at 100 mg/kg bw/day and 6 top dose group females. This finding was observed for one day only and all these animals had normal pregnancies, therefore, it was considered not to be of toxicological significance. Three of these animals had no resorptions and the remainder had 1-4 resorptions.

Reduced body weight gains were noted in the top dose group during treatment and mean body weights were about 94-96% of the control value during the period of dosing. There was a dose related and statistically significant reduction in food consumption in the 100 and 1000 mg/kg bw/day dose groups during the treatment period. At 1000 mg/kg bw/day food consumption was 70 and 85% of control values at days 6-11 and 11-16 respectively and at 100 mg/kg bw/day 92% for both periods.

Pregnancy status was not affected by treatment. The number of dams with viable fetuses at scheduled sacrifice was 23/24, 22/24, 20/24 and 22/24 at 0, 10, 100, and 1000 mg/kg bw/day respectively. Necropsy revealed no further macropathological findings in treated animals. Preimplantation losses, number of implantation sites and early and late implantation losses were comparable between groups. No dead or aborted fetuses were noted. Numbers of live fetuses per litter and foetal weights were not affected by treatment. Necropsy of the dams revealed no macroscopically observable pathological changes.

External examination revealed no treatment related abnormalities.

The only apparently treatment related finding from visceral examination was an enlarged thymus (considered a variation) seen in 3-3-3-11 fetuses in the concurrent control, low, mid, and top dose group. This incidence was statistically significant and slightly outside the historical control range. However, it is considered to be of minimal toxicological significance.

No skeletal malformations were observed in this study. The skeletal anomalies observed consisted of fused or asymmetric sternebrae, irregular ossification of the cranial bones, poor ossification of metacarpal-, additional cervical vertebral arches and bipartite thoracic vertebral centres. There were no treatment related effects on the incidence of these skeletal anomalies or variations.

In conclusion maternal toxicity was evident at 1000 mg/kg bw/day based on effects on bodyweight and food consumption. Although food consumption was marginally affected at 100 mg/kg bw/day, in the absence of other findings this dose level is considered the NOAEL for maternal toxicity. The NOAEL for fetotoxicity was 100 mg/kg bw/day based on the increased incidence of enlarged thymus found in the 1000 mg/kg bw/day group.

After discussion at the EU Pesticides Peer Review teleconference 144, the maternal NOAEL was reduced to 10 mg/kg bw/day based on the decreased body weight gain and food consumption observed at higher doses. The developmental NOAEL remained at 100 mg/kg bw/day (EFSA conclusion on pesticides peer review, 2017-09-14).

10.10.4.2 Developmental toxicity in rabbits

In a preliminary range finding developmental study, trifloxystrobin was administered to five groups of five artificially inseminated Russian (Chbb: HM) rabbits by gavage daily from day 7 through 19 of gestation at dose levels of 0, 20, 100, 500 and 1000 mg/kg bw/day. There were no deaths at any dose. Clinical signs such as reduced activity and haemorrhagic discharge occurred at 1000 mg/kg bw/day. Dose dependent effects on food consumption and body weight were observeded at doses ≥ 100 mg/kg bw/day. At 500 mg/kg bw/day food consumption was consistently decreased by up to 77% throughout the treatment period, and as a result, body weight losses occurred. The number of post implantation losses (1 dam with total resorptions) was increased and the number of foetuses decreased. Foetal body weight was reduced at 500 mg/kg bw/day. At 1000 mg/kg bw/day all pregnant animals had total resorptions, food consumption and body weight data were not reported. Based on these results, 500 mg/kg bw/day was selected as high dose level for the main developmental toxicity study with trifloxystrobin.

Groups of 17-19 presumed-pregnant Russian (Chbb:HM) rabbits were administered trifloxystrobin (purity 96.4%) by gavage in 0.5% aqueous Na-carboxymethylcellulose at doses of 0, 10, 50, 250 and 500 mg/kg bw/day from days 7 to 19 of gestation. Dose levels were based on a range finding study. Measurements of bodyweight, food consumption and an assessment of clinical signs were made regularly. The animals were sacrificed on day 29 of gestation, macroscopic pathological changes in maternal organs noted, and the ovaries and uteri examined. Fetuses were weighed and examined for visceral and skeletal abnormalities.

No treatment related mortality or clinical signs occurred. One dam of the 50 mg/kg bw/day dose group died spontaneously without having exhibited any clinical signs before death. At necropsy haemorrhagic contents of uterus was found with this animal.

At doses ≥ 250 mg/kg bw/day there was a dose related reduction in bodyweight gain and a significant bodyweight loss during the treatment period. Reduced food consumption was associated with these findings.

Pregnancy status was not affected by treatment. The number of dams with viable fetuses at scheduled sacrifice was 19/19, 18/19, 16/19, 17/19 and 18/19, at 0, 10, 50, 250 and 500 mg/kg bw/day respectively. Necropsy revealed no further macropathological findings in treated animals. The number of corpora lutea, pre-implantation losses, numbers of implantation sites, and post-implantation losses were comparable between groups. There were no dead or aborted fetuses in any group. The numbers of live fetuses per litter and fetal weights were unaffected by treatment.

Table 13: Summary of total malformation, anomalies[a] and variations – rabbit study

Dose (mg/kg bw/d)		**0**	**10**	**50**	**250**	**500**
Litters evaluated	[N]	19	18	16	17	18
Fetuses evaluated	[N]	116	130	90	97	97
Live	[N]	116	130	90	97	97
Dead	[N]	0	0	0	0	0
Total malformations						
Fetal incidence	No. (%)	0 (0.0)	1 (0.8)	2 (2.2)	1 (1.0)	3 (3.1)
Litter incidence	No. (%)	0 (0.0)	1 (5.6)	2 (12.5)	1 (5.9)	3 (16.7)
Affected fetuses / litter	%	0.00 ± 0.00	0.62 ± 2.62	1.79 ± 4.88	0.98 ± 4.04	2.73 ± 6.64
Total anomalies[a,b]						
Fetal incidence	No. (%)	12 (10.3)	13 (10.0)	9 (10.0)	24 (24.7)	22 (22.7)
Litter incidence	No. (%)	8 (42.1)	7 (38.9)	7 (43.8)	12 (70.6)	9 (50.0)
Affected fetuses / litter	%	9.99 ± 13.69	9.71 ± 14.42	10.84 ± 15.19	23.94 ± 18.38	23.16 ± 29.32
Total variations						
Fetal incidence	No. (%)	98 (84.5)	107 (82.3)	74 (82.2)	80 (82.5)	77 (79.4)
Litter incidence	No. (%)	19 (100)	18 (100)	16 (100)	16 (94.1)	17 (94.4)
Affected fetuses / litter	%	84.06 ± 19.65	80.01 ± 24.71	81.39 ± 24.64	76.36 ± 28.76	76.29 ± 28.63

Statistical analysis: Litter incidence: Chi-square + Fisher's Exact test; affected fetuses/litter: Kruskal-Wallis + Mann-Whitney U-test; * $p \leq 0.05$, ** $p \leq 0.01$

[a] Note: according to the study report Anomaly is defined as: rare, slight to moderate, permanent or reversible structural change that is not considered to impair fetal survival, development or function.

[b] Increased total anomalies at 250 and 500 mg/kg bw/d attributed to the increased incidence of skeletal anomalies

The nature and incidences of external and visceral abnormalities did not indicate an effect of treatment at any dose level.

At external fetal examination two fetuses with malformations were seen; a single fetus of the low dose group showed craniocele and at 250 mg/kg bw/day a fetus exhibited the following malformations; gastrochisis, acromicria and ectodactyly of the left forelimb. Since all these findings occurred in single individuals only, without any dose dependency or statistical significance, they were not considered treatment-related.

Forelimb position anomaly (unilateral) was evenly distributed among all treated groups. There was no dose relationship and the group incidences were within historical ranges with the exception of the fetal incidence at 250 mg/kg bw/day, which slightly exceeded the historical control range; historical database of 2564 fetuses and 456 litters (fetal % incidence/litter % incidence ranges: 0.0-2.5%/0.0-13.3%). Thus this finding is considered unlikely to be treatment related. The study authors considered that this anomaly (flexure of the forepaw at the wrist) was most likely due to restriction of movement in the uterus and in the absence of related morphological findings, it is not categorised as a malformation[1].

Fetal visceral examinations revealed no treatment related or toxicologically significant findings. A visceral malformation - aplasia of the gall bladder - occurred in one low-mid and in two high dose fetuses. In the expert opinion of the study director, this finding was not treatment-related.

As detailed in Table 14, skeletal malformations were observed in a low dose fetus (reduced interparietal, parietal, frontal and nasal bones), a 50 mg/kg bw/day fetus (reduced interparietal bone), a 250 mg/kg bw/day fetus (absent ossification of ulna), and a high dose fetus (absent ossification of pubis). All these malformations were considered to be spontaneous and not related to dosing with trifloxystrobin.

Table 14: Summary table of fetal skeletal malformations – rabbit study

Finding	**Dose level (mg/kg bw/day) Fetal % incidence (litter % incidence)**				
	0	**10**	**50**	**250**	**500**
Total fetuses examined (litters examined)	116 (19)	130 (18)	90 (16)	97 (17)	97 (18)
Reduced interparietal bone	-	0.8 (5.6)	1.1 (6.3)	-	-
Reduced parietal bone	-	0.8 (5.6)	-	-	-
Reduced frontal bone	-	0.8 (5.6)	-	-	-
Reduced nasal bone	-	0.8 (5.6)	-	-	-
Fore limb – absent ossification ulna	-	-	-	1.0 (5.9)	-
Fore paw - adactyly	-			1.0 (5.9)	-
Pelvic girdle – absent ossification pubis	-	-	-	-	1.0 (5.6)
Total skeletal malformations	0 (0.0)	0.8 (5.6)	1.1 (6.3)	1.0 (5.9)	1.0 (5.6)

[1] Palmer, A.K. (1978). Developmental Abnormalities: Rabbits, in: Pathology of Laboratory Animals, Vol. II; Springer Verlag, New York, Chapter 20, p. 1855

As detailed in Tables 15 and 16, skeletal anomalies observed in the fetuses consisted mainly of fused or asymmetric sternebrae. Fragmented sternebrae, irregular ossification of scapula, and displaced cervical and caudal vertebral centres occurred in addition but without showing any dose dependency.

Table 15: Fetuses with fused sternebrae – rabbit study

Parameter		**Dose (mg/kg bw/day)**					
		0	**10**	**50**	**250**	**500**	**HCD**
No. fetuses evaluated		116	130	90	97	97	2562
No. litters evaluated		19	18	16	17	18	455
Sternebra(e)							
Sternebra 1, fused 1 and 2	Fetuses affected [N]	1	1	1	2	1	0 – 2
	Fetal incidence [%]	0.9	0.8	1.1	2.1	1.0	0.0 – 2.5
	Litter incidence [%]	5.3	5.6	6.3	**11.8**	5.6	0.0 – 10.5
Sternebra 2, fused 2 and 3	Fetuses affected [N]	1	1	1	4	4	0 – 5
	Fetal incidence [%]	0.9	0.8	1.1	4.1	4.1	0.0 – 5.7
	Litter incidence [%]	5.3	5.6	6.3	**23.5**	**22.2**	0.0 – 20.0
Sternebra 3, fused 3 and 4	Fetuses affected [N]	2	2	1	5	**10***	0 – 8
	Fetal incidence [%]	1.7	1.5	1.1	5.2	**10.3**	0.0 – 9.2
	Litter incidence [%]	10.5	5.6	6.3	23.5	33.3	0.0 – 33.3
Sternebra 4, fused 4 and 5	Fetuses affected [N]	4	2	4	7	**8**	0 – 7
	Fetal incidence [%]	3.4	1.5	4.4	7.2	**8.2**	0.0 – 8.0
	Litter incidence [%]	21.1	11.1	25.0	**35.3**	**33.3**	0.0 – 29.4

Statistical analysis: Chi-square + Fisher´s Exact test; * $p \leq 0.05$

Values exceeding HCD are written in **bold letters**

HCD (revised supplement, 1999): 20 studies (24 control groups) performed at the test facility (1989–1995) with Russian Chbb:HM rabbits (455 litters with 2562 viable fetuses examined)

Table 16: Fetuses with asymmetrically shaped sternebrae – rabbit study

Parameter		Dose (mg/kg bw/day)					
		0	**10**	**50**	**250**	**500**	**HCD**
No. fetuses evaluated		116	130	90	97	97	2562
No. litters evaluated		19	18	16	17	18	455
Sternebra(e)							
Sternebra 1, asymmetrically shaped	Fetuses affected [N]	0	1	1	2	**3**	0 – 2
	Fetal incidence [%]	0.0	0.8	1.1	2.1	**3.1**	0.0 – 2.3
	Litter incidence [%]	0.0	5.6	6.3	5.9	5.6	0.0 – 13.3
Sternebra 2, asymmetrically shaped	Fetuses affected [N]	0	1	1	2	**4**	0 – 3
	Fetal incidence [%]	0.0	0.8	1.1	2.1	4.1	0.0 – 4.1
	Litter incidence [%]	0.0	5.6	6.3	11.8	**16.7**	0.0 – 13.3
Sternebra 3, asymmetrically shaped	Fetuses affected [N]	0	1	0	2	3	0 – 3
	Fetal incidence [%]	0.0	0.8	0.0	2.1	**3.1**	0.0 – 2.7
	Litter incidence [%]	0.0	5.6	0.0	**11.8**	**16.7**	0.0 – 10.5
Sternebra 4, asymmetrically shaped	Fetuses affected [N]	0	1	0	4	2	0 – 4
	Fetal incidence [%]	0.0	0.8	0.0	**4.1**	2.1	0.0 – 3.2
	Litter incidence [%]	0.0	5.6	0.0	**23.5**	11.1	0.0 – 17.6
Sternebra 5, asymmetrically shaped	Fetuses affected [N]	0	0	0	0	1	0 – 3
	Fetal incidence [%]	0.0	0.0	0.0	0.0	1.0	0.0 – 2.7
	Litter incidence [%]	0.0	0.0	0.0	0.0	5.6	0.0 – 17.6
Sternebra 6, asymmetrically shaped	Fetuses affected [N]	0	0	1	1	0	0 – 2
	Fetal incidence [%]	0.0	0.0	1.1	1.0	0.0	0.0 – 2.3
	Litter incidence [%]	0.0	0.0	6.3	5.9	0.0	0.0 – 13.3

Statistical analysis: Chi-square + Fisher´s Exact test; * $p \leq 0.05$
Values exceeding HCD are written in **bold letters**
HCD (revised supplement, 1999): 20 studies (24 control groups) performed at the test facility (1989–1995) with Russian Chbb:HM rabbits (455 litters with 2562 viable fetuses examined)

The incidence of fused sternebrae and asymmetrically shaped sternebrae was slightly increased in the two higher dose groups. For some anomalies of single segments the incidence was slightly outside the control range. Statistical significance was reached only for the occurrence of fused sternebrae-3 and -4 in the high dose group and was considered to be possibly treatment-related.

Based on the increased incidences of the sternebral findings, the EU Pesticides Peer Review teleconference 144 meeting proposed a classification for reproductive toxicity category 2. With regard to that, the applicant presented more details of these findings based on the individual fetal data in the study report (Table 17). These data revealed that severe sternebral fusion, alignment of ribs with the sternebrae or abnormal curvature of the sternum, which could possibly impair post-natal development resulting in a shortened rib cage with consequently impairment of further pup development, did not occur. Findings observed consisted of fusion of adjacent sternebrae observed in 2-1-1-3-3 fetuses after 0-10-50-250-500 mg/kg bw/day and partial fusions of adjacent sternebrae observed in 3-3-3-8-11 fetuses. The more severe finding of fusion and/or partial fusion of all segments of the sternum (sternebrae 1 to 5) occurred in one fetus each in all groups except of the 10 mg/kg bw/day group. The sternal findings were observed in 5-3-4-8-6 litters at 0, 10, 50, 250, 500 mg/kg bw/day without showing a dose response.

Table 17: Details of sternal findings based on individual data in the study report

Parameter		**Dose (mg/kg bw/day)**				
		0	**10**	**50**	**250**	**500**
No. fetuses		116	130	90	97	97
No. litters evaluated		19	18	16	17	18
Sternebra(e)		***[identification number dam/identification number fetus]***				
Sternebra 1 and 2	Fused	7/8	25/6		63/7	
	Partially fused			55/1	65/6	93/1
Sternebra 2 and 3	Fused				63/7	93/1
	Partially fused	7/8	25/6	55/1	65/4, 66/7, 74/4	84/1, 86/3, 87/5
Sternebra 3 and 4	Fused	7/8	25/6	55/1		93/5
	Partially fused	11/5	25/3		63/1, 63/7, 66/7, 67/7, 74/4	82/3, 84/2, 85/5, 86/3, 87/5, 93/1, 93/2, 93/6, 93/8
Sternebra 4 and 5	Fused	7/8, 9/1		55/1	66/7, 70/4	82/3, 93/1
	Partially fused	10/1, 17/2	32/7, 36/1	39/7, 48/2, 51/9	62/3, 63/7, 71/1, 71/7, 74/4	84/7, 85/4, 86/3, 86/7, 87/5, 93/6
Asymmetrically shaped			25/6	55/1	62/3, 63/1, 63/7, 63/8, 72/4, 74/4	86/3, 87/5, 93/1, 93/5, 93/6
No. of fetuses with						
Fused sternebra(e)		2	1	1	3	3
Partially fused sternebra(e)		3	3	3	8	11
Fusion of sternebrae 1-5		1	0	1	1	1

Skeletal examination of fetuses in developmental toxicity studies represents a single 'snapshot' in time; hence, an appreciation of the sequence and normal patterns of ossification aids in the differentiation of generalised delays and minor alterations from true skeletal dysplasia. In rodents and rabbits, the sternebrae are amongst the regions that ossify rapidly during late gestation: sternebra 1 ossify first, followed by sternebra 2 , 3 , 6 , 4 with sternebra 5 being last. Sternebral ossification is an on-going process starting perinatally and in rabbits it is finalised by an age of 3 months[2]. Variable ossification of these late-ossifying bones is normal in rodents and rabbits, with the incidence of fetuses with ossification in these sites being dependent upon the day of gestation at sacrifice and the criteria used by each laboratory for individual bones. Small premature sternal fusions and asymmetrically (ossified) shaped sternebrae at the end of the gestation period are not considered to adversely affect post-natal development.

Alterations of sternal elements (e.g. unossifications, fusions, asymmetric shape) are amongst the most commonly occurring developmental variations in Russian rabbits. Historical control data on Russian rabbits presented in the study report and as compiled by the Applicant in addition show that these sternal findings are relatively common in this strain (please see Appendix 1).

A more in depth analysis of fetal body weights and sternal findings has been conducted by the Applicant and is presented in Appendix 2. This analysis supports the view that maternal toxicity excacerbated the background incidence of sternal findings.

In conclusion maternal toxicity was evident at ≥ 250 mg/kg bw/day based on effects on bodyweight and food consumption. There was no treatment-related increase in the incidence of malformations. There was an apparent increased incidence of skeletal (sternal) variations at ≥ 250 mg/kg bw/day although statistical significance was only achieved at the top dose. The variations seen in the sternebrae only occurred at dose levels with maternal toxicity (body weight losses and markedly reduced food consumption < 50% of control

[2]Kamel B.M., Rashed R.F., Erasha A.M. (2016): Development of sternum and ribs in White New Zealand Rabbits (Orictolagus cuniculus). World Vet. J. 6(3) pp. 143-150

values during the treatment period). Based on these effects the NOAEL for maternal and developmental toxicity was 50 mg/kg bw/day (EFSA conclusion on pesticides peer review, 2017-09-14).

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

The potential for trifloxystrobin to cause developmental toxicity has been investigated in two guideline studies; one in rats, and one in rabbits. Information on developmental toxicity is also available from 2 generation study on rats.

In the two generation study, pre-natal and post-natal viability was not affected at any dose (up to 1500 ppm). Toxicity to the pups consisted of reduced body weight and body weight gain during the lactation period at 750 and 1500 ppm. At the top dose, pup body weights were lower than controls by PND4, although the effect was slight (<10%) and not statistically significant. By PND7, body weights were significantly lower than controls (by up to 16%) in both sexes of each generation. By PND14, pup weights were up to 22% lower than controls, and by PND 21 (i.e., the end of the lactation period), pup weights were up to 28% lower than controls. At 750 ppm, significant effects on body weight were observed from PND14 in the F1a and F1b pups, and from PND7 in the F2 pups. By the end of the lactation period, body weights were 9%, 11% and 14% lower in the F1a, F1b and F2 pups respectively (mean values for males and females).

The cause of the reduced body weights in pups is not clear. Published data suggests that the pups start to consume solid food on PND12, and that suckling is complete by day 21[3]. It is therefore possible that reductions in body weight from PND12 onwards are at least partly due to the consumption of treated diet. Alternatively, pups may have avoided the treated diet if there were issues with palatability, leading to reductions in body weight compared to controls.

However, the effects on body weight seen on PND7 at 1500 ppm cannot be attributed to the consumption of treated diet. There is no evidence to suggest that trifloxystrobin or its metabolites are transferred into the milk, and no data are available on the quality or quantity of milk produced by the mothers in this study (see Section 10.10.7). It is possible that the effects on pup weight are a non-specific secondary effect of maternal toxicity.

The effect on pup weight does not appear to have had any long term adverse effects in these animals (i.e., the pups survived to adulthood and produced viable offspring). The pups developed into adults whose body weights were lower than controls throughout the course of the study (consistent with the lower level of food consumption in these animals), however the birthweights of their offspring were comparable to controls.

At 1500 ppm, retardation in the eye opening landmark during both lactation periods in the F1 generation and the F2 generation were observed, although this is likely to be related to the reduced body weights in these animals. There was no evidence of a specific effect on development in this study.

In a developmental study in rats, maternal toxicity was evident at doses of ≥100 mg/kg bw/day based on reductions in bodyweight and food consumption. There were no treatment-related effects on skeletal anomalies or variations, and the only visceral observation of note was an increased incidence of enlarged thymus at 1000 mg/kg bw/day. However, this finding is considered a variation and in isolation is considered not to be of toxicological significance. Furthermore, it was only observed at a dose level exhibiting maternal toxicity. Overall, it is concluded that trifloxystrobin exhibited no significant developmental toxicity in the rat.

In a rabbit developmental toxicity study, maternal toxicity was evident at doses ≥ 250 mg/kg bw/day based on effects on body weight and food consumption (body weight losses and markedly reduced food consumption during the treatment period). An increased incidence of skeletal (sternal) findings was reported at doses ≥ 250 mg/kg bw/day, achieving statistical significance at the top dose for single sternebrae. Additional analysis of

[3] Pérez-Cano FJ, Franch À, Castellote C and Castell M (2012) The suckling rat as a model for immunonutrition studies in early life. *Clinical and Developmental Immunology*, Volume 2012 (2012), Article ID 537310

the individual foetal data showed that severe sternebral fusions were not reported. The number of foetuses with fusions of all segments of the sternum (sternebrae 1-5) were evenly distributed in all groups. There were some differences from controls in the foetal incidence of partially fused and asymmetrically shaped sternebrae at doses ≥ 250 mg/kg bw/day. As shown by the historical control data for this rabbit strain, these are very common variations which represent only small deviations from the normal situation i.e. a change that occurs within the normal population under investigation and is unlikely to adversely affect survival or health[4]. Overall, it is concluded that trifloxystrobin exhibited no significant developmental toxicity in the rabbit.

10.10.6 Comparison with the CLP criteria

There is no data on humans to inform on the developmental toxicity of trifloxystrobin, and thus classification in category 1A is not appropriate.

Classification in category 1B for developmental toxicity is not appropriate as there is no clear evidence of an adverse effect on development in experimental animals.

Substances are classified in category 2 when there is some evidence from humans or experimental animals of an adverse effect on development, and where the evidence is not sufficiently convincing to place the substance in category 1. Furthermore, the 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.

In a 2-generation study, there were no effects on embryo or fetal lethality, or on pup survival during lactation and weaning. At doses ≥ 750 ppm, pup body weights were reduced compared to controls during the lactation period. At 750 ppm, the effect was statistically significant from PND14 in the F1a and F1b pups, and from PND7 in the F2 pups. At 1500 ppm, the effect was statistically significant from PND7 in the Fla, F1b and F2(male) pups, and from PND4 in the F2 females.

The dossier submitter considers that the retarded body weight development in the pups during the lactation period was likely to be a non-specific secondary effect of maternal toxicity (i.e., reduced food consumption and lower body weights during gestation and lactation in the parental females). A direct effect arising from pups consuming treated diet during weaning was also likely to be a contributing factor. Minor delays in eye opening are considered secondary to the retarded pup body weight development.

In the rat developmental toxicity study the only observation of note was an increased incidence of enlarged thymus in the presence of maternal toxicity. This finding is a variation and in isolation is considered not to be of toxicological significance. Thus, this minor developmental change does not meet the criteria for classification.

In the rabbit developmental toxicity study an increased incidence of skeletal (sternal) findings was observed in the presence of maternal toxicity. In the summary tables in the Draft (Renewal) Assessment Report the incidences of these findings were presented and analysed statistically as fusions of adjacent bones and as asymmetrically shaped sternebrae. However, in cases where more than two sternebrae were affected, it was not possible to assess exactly how many foetuses and litters per dose group were affected. Furthermore, no indication of the severity of the fusions was provided. Thus, a classification of the finding as malformation or variation was not possible. Based on the presented data, the EU Pesticides Peer Review teleconference 144 meeting proposed a classification for reproductive toxicity category 2 (EFSA conclusion on pesticides peer review, 2017-09-14).

[4] Chahoud I, Buschmann J, Clark R, Druga A, Falke H, Faqi A et al. (1999). Classification terms in developmental toxicology: need for harmonisation . Report of the Second Workshop on the Terminology in Developmental Toxicology Berlin, 27-28 August 1998. Reprod. Toxicol. 13:77-82.

An analysis of the individual foetal data showed that severe sternebral fusion, alignment of ribs with the sternebrae or abnormal curvature of the sternum, which could possibly impair post-natal development resulting in a shortened rib cage with consequently impairment of further pup development, did not occur. There was also no evidence of a treatment-related effect of fusion of all segments of the sternum (sternebrae 1 to 5). Actually, partially fused and asymmetrically shaped sternebrae occurred which represent small deviations from normal sternal development, common variations that have no effect on survival and do not persist post-natally. The findings occurred only in the presence of marked maternal toxicity. There were no other skeletal or visceral adverse changes and no embryo-foetal deaths that could have masked an increase in foetal abnormalities. No sternebral findings were seen in the rat developmental toxicity study up to the limit dose of 1000 mg/kg bw/day, twice the top dose of the rabbit developmental study. Thus, this minor developmental change does not support classification.

In conclusion, trifloxystrobin does not meet the criteria for classification for developmental toxicity in these studies.

10.10.7 Adverse effects on or via lactation

In the 2 generation study in rats, a treatment-related reduction in pup body weight was observed during the lactation periods of each generation (see Section 10.10.1). No data are available on the quality or quantity of milk produced by the mothers in this study. Furthermore, the milk in this study was not analysed for the presence of trifloxystrobin or its metabolites.

Three studies are available conducted in lactating ruminants (two in goats, one in cows) designed to investigate whether trifloxystrobin or its metabolites are transferred to the milk. These studies are summarised in Table 18.

Table 18: Summary table of other studies relevant for effects on or via lactation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Metabolism in lactating goat	[Trifluoromethyl-phenyl-U-^{14}C] trifloxystrobin (TP)	2 Lactating goats were given 4 x daily doses of 4.24 mg/kg bw by capsule equivalent to 103.8 ppm in feed. Animals sacrificed 6 h after last dose. Milk, urine, faeces and cagewash collected daily. Bile, blood, musce , fat, liver and kidney taken at sacrifice. Radioacitivity measured in excreta, milk and tissues. Where possible metabolite profile in excreta, milk and tissues were identified.	A total of 0.08%, 44.5% and 17.4% of the dose was excreted via milk, faeces and urine, respectively. Averaged radioactive residues were lower in milk than blood or bile; 0.085, 0.2248 and 7.131 mg/kg, respectively. Unchanged parent was the major component in milk accounting for 51.6% radioactivity in milk. The next most aboundant component was the taurine conjugate of acid metabolite CGA 321113 and accounted for 13% radioactivity. One characterised metabolite accounted for 11% radioactivity and the remaining 6 metabolites each accounted for <5% radioactivity in milk. The overall metabolic pathway of trifloxystrobin is similar in goat and rat.	Anonymous (1997a) , M-034501-01-1
Metabolism in lactating goat	[Glyoxyl-phenyl-U-^{14}C] trifloxystrobin (GP),	2 Lactating goats were given 4 x daily doses of 4.13 mg/kg bw by capsule equivalent to 100.4 ppm in feed. Animals sacrificed 6 h after last dose. Milk, urine, faeces and cagewash collected daily. Bile, blood, muscle , fat, liver and kidney taken at sacrifice. Radioacitivity measured in excreta, milk and tissues. Where possible metabolite profile in excreta, milk and tissues were identified.	A total of 0.06%, 36.0% and 18.9% of the dose was excreted via milk, faeces and urine, respectively. Radioactive residues were lower in milk than blood or bile; 0.089, 0.330 and 40.813 mg/kg, respectively. Unchanged parent was the major component in milk accounting for 73.8% radioactivity in milk. Acid metabolite CGA 321113 and its taurine conjugate were also detected accounting for 3-4% and 6 other metabolites each accounted for <5% radioactivity in milk. The overall metabolic pathway of trifloxystrobin is similar in goat and rat.	Anonymous (1997b), M-034517-01-1

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Magnitude of the residues in meat and milk resulting from feeding of three dose levels to cattle	Trifloxystrobin.	Trifloxystrobin was administered orally (via capsule) to 3 lactating cows/group for 28-30 consecutive days at average dose rates of 2.0, 5.9 and 21 mg/kg feed dry matter corresponding to 0.065, 0.193 and 0.635 mg/kg bw/day, respectively. A control animal received an empty capsule. Animals were sacrificed 20-24 hours after the last dose. Milk samples were collected pre-dose and at 6 intervals throughout the study. At sacrifice blood and tissue samples were taken. Samples were analysed for trifloxystrobin and its acid metabolite CGA 321113 using a validated gas chromatography method with nitrogen-phospohorous detection.	During the course of the study, no residues of trifloxystrobin (CGA 279202) and its acid metabolite CGA 321113 were determined above the LOQ of 0.01 mg/kg in the milk from any sampling event and of any dose group.	Anonymous (1997c), M-038221-01-1

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

The studies conducted in lactating goats and cows indicate that very low levels of trifloxystrobin or its metabolites are present in milk. In the goat, dosed with 4.1-4.2 mg/kg bw, the dominant elimination was via faeces and only 0.06-0.08% of the total dose was eliminated via milk which corresponds to 0.085-0.089 ppm trifloxystrobin equivalents. It is noted that a plateau of radioactivity in milk was not definitively achieved following 4 days of dosing but given the exceedingly low levels of radioactivity present this is considered not to affect the conclusions of the study. In the cow, following 28-30 consecutive days of oral dosing of up to 0.635 mg/kg bw, there was no trifloxystrobin nor the metabolite CGA 321113 detected at above the LOQ of 0.01 mg/kg in milk.

Overall, it can be concluded that trifloxystrobin is not excreted in the milk to any appreciable extent in cows or goats.

10.10.9 Comparison with the CLP criteria

Under CLP, substances that are absorbed by women and have been shown to interfere with lactation shall be classified and labelled to indicate this property hazardous to breastfed babies. Effects in the mother can adversely impact the breast milk (either in terms of the quantity produced or the quality produced). However, if a substance causes overt toxicity in the mother, this may indirectly impair milk production or impair maternal care as a non-specific secondary effect and should not lead to classification.

a) Human evidence indicating a hazard to babies during the lactation period

No data from humans are available.

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

In the 2 generation study in rats, a treatment-related reduction in pup body weight was observed during the lactation periods of each generation. No data are available on the quality or quantity of the milk produced by the mothers in this study, and the milk was not analysed to identify whether trifloxystrobin or its metabolites were present. However, based on the available tokicokinetic data, and data from lactating goats and cows, it is considered highly unlikely that trifloxystrobin or its metabolite are transferred into the milk. It is more likely that the reduced body weights in pups were partly due to the consumption of treated diet from PND12 (or avoidance of treated diet, on the basis of palatability). Reductions in body weight prior to PND 12 were likely to be a non-specific secondary effect of maternal toxicity, as evidenced by reduced body weights, food consumption and slight kidney/liver toxicity in the mothers. In conclusion, there is no clear evidence of an adverse effect due to transfer in the milk, or an adverse effect on the quality of the milk.

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

The available toxicokinetic data suggest that trifloxystrobin is rapidly metabolised to more polar, water soluble molecules which are excreted primarily via the urine and bile. On this basis, it is unlikely that the metabolites would be transferred to the milk. It is noted that trifloxystrobin has a log POW of 4.5, suggesting that it may have some potential for transfer to milk. However, this is not supported by studies conducted in lactating goats and cows, which show negligible transfer of trifloxystrobin to the milk.

Based on the above assessment and comparison with the classification criteria, trifloxystrobin does not meet the criteria for classification for effects on or via lactation.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Not classified. Data conclusive but not sufficient for classification.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Sexual function and fertility

The potential of trifloxystrobin to adversely affect sexual function and fertility was investigated in a 2-generation study in rats, preceded by a 1-generation range-finding study.

In the range finding study (non-guideline, non-GLP, Anonymous, 1995), trifloxystrobin was administered via the diet to groups of 15 rats/sex/dose at dietary concentrations of 0, 100, 1 000 or 2 000 ppm (equal to 0, 6, 53.5 and 109.6 mg/kg bw/d for males and 0, 7.9-16.5, 67.0-168.6 and 140.5-321.7 mg/kg bw/d for females). Food intake and body weight and body weight gain were reduced during treatment in the parental animals of the mid and high dose groups (food consumption 0-14 % and 0-15 %, respectively; body weight 0-5 % and 1-8 %, respectively). Mortality, clinical signs or remarkable observations at gross necropsy were not observed. Reproduction was not affected at any dose. At 2 000 ppm, pup body weight was

statistically significantly decreased at lactation day (LD) 7 (by 12 %) and LD14 (by 15 %), with a decrease of 19 % over LD0-14. Based on the parental and offspring findings, 1 500 ppm was recommended as high dose level for a 2-generation study.

In the 2-generation study (OECD TG 416, GLP, Anonymous, 2001), groups of 30 rats/sex/dose were administered trifloxystrobin in the diet at concentrations of 0, 50, 750 or 1 500 ppm (equal to 0, 2.3-3.8, 32.9-58.4 and 73.1-126.7 mg/kg bw/d for males and 0, 3.1-8.0, 47.9-119.9 and 98.0-242.0 mg/kg bw/d for females). There were no treatment-related deaths or clinical signs among the F0 and F1 parental animals. Male and female reproductive parameters and organs were not affected by treatment. Parental toxicity at the mid and high dose consisted of slight reductions in food consumption and body weight as compared to controls at several time points during pre-/post-mating, gestation and lactation, and slight toxicity of the liver and kidneys. The decrease in food consumption was generally less than 10 %, but occasionally it was up to 16 % (F0) or 25 % (F1), mainly during the first week(s) of pre-mating and the third week of lactation. Decreases in body weight mirrored these, and were up to 8 %/11 % in mid dose F0/F1, 5-13 % in high dose F0, and 15-20 % (but during the first weeks of pre-mating up to 30 %) in high dose F1. Pup toxicity was evident at these doses as retarded weight development during lactation (statistically significant from LD7 onwards), and F1a, F1b and F2 pups also showed a delay in eye opening of 0.6-0.7 days at the high dose.

In the absence of treatment-related effects on fertility, reproduction and pregnancy outcome, the dossier submitter (DS) concluded that trifloxystrobin does not meet the criteria for classification.

Developmental toxicity

The developmental toxicity of trifloxystrobin was investigated in rats and rabbits.

Rats

In a guideline study (OECD TG 414, GLP, Anonymous, 1999a), pregnant Tif:RAIf (SPF) rats (20-23/dose) received trifloxystrobin (in 0.5 % aqueous sodium carboxymethylcellulose) orally by gavage at dose levels of 0, 10, 100 or 1 000 mg/kg bw/d from gestation day (GD) 6-15. The dose levels in this study were chosen based on the findings in a preliminary range finding developmental study (7 pregnant Tif:RAIf (SPF) rats/dose, non-guideline, non-GLP, Anonymous, 1993), where there was no toxicity at 10 and 100 mg/kg bw/d, and 1 000 mg/kg bw/d resulted only in slight reductions in maternal food consumption and body weight gain throughout the treatment period. In the main study, there were no mortalities. Dams showed reduced food consumption (at mid and high dose) and body weight and bodyweight gain (at high dose) during the treatment period, resulting in a net weight change (weight gain over GD6-21 minus gravid uterus weight) that was 18 % and 32 % lower at the mid and high dose, respectively. Pregnancy status, numbers of implantations sites, pre-/post-implantation losses and live foetuses per litter were not affected by treatment, nor were foetal weights. No dead or aborted foetuses were noted, and external and skeletal examination revealed no treatment-related malformations, anomalies or variations. Upon visceral examination, the only apparently treatment-related finding was enlarged thymus in the high dose group, with a foetal incidence of 7.5 % and a litter incidence of 31.8 %. Although the increased foetal incidence reached statistical significance and both the foetal and litter incidences were slightly outside the historical control data (HCD) ranges (0-6.0 % and 0-29.2 %, respectively), the DS considered this finding a variation and in isolation not to be of toxicological significance. It therefore does not meet the criteria for classification.

Regarding the retarded body weight development in the pups during the lactation period in the 2-generation study, the DS considered that likely to be a non-specific secondary effect of maternal toxicity (i.e., reduced food consumption and lower body weights during gestation and lactation in the parental females). A direct effect arising from pups consuming treated diet during weaning was also likely to be a contributing factor. The DS considered the minor delays in eye opening secondary to the retarded pup body weight development. As there was no evidence of a specific effect on development in this study, and the effect on pup weight did not appear to have had any long term adverse effects in these animals (i.e., the pups survived to adulthood and produced viable offspring), classification was not considered warranted.

Rabbits

In a preliminary range finding developmental study (non-guideline, non-GLP, Anonymous, 1994a), trifloxystrobin was administered by gavage to pregnant Russian (Chbb:HM) rabbits (5/group) at dose levels of 0, 20, 100, 500 or 1 000 mg/kg bw/d from GD7-19. There were no deaths at any dose. Clinical signs such as reduced locomotor activity and haemorrhagic discharge occurred at 1 000 mg/kg bw/d. Dose dependent effects on food consumption and body weight were observed at doses from 100 mg/kg bw/d, at 500 mg/kg bw/d resulting in body weight loss throughout the treatment period. At 500 mg/kg bw/d, the number of post-implantation losses (1 dam with total resorptions) was increased, the number of foetuses was decreased and foetal body weight was reduced. At 1 000 mg/kg bw/d, all pregnant animals had total resorptions; food consumption and body weight data were not reported. Based on these results, 500 mg/kg bw/d was selected as high dose level for the main developmental toxicity study with trifloxystrobin.

In the main study (OECD TG 414, GLP, Anonymous 1999b), pregnant Russian (Chbb:HM) rabbits (17-19/dose) received trifloxystrobin (in 0.5 % aqueous sodium carboxymethylcellulose) orally by gavage at dose levels of 0, 10, 50, 250 or 500 mg/kg bw/d from GD7-19. No treatment-related mortality or clinical signs occurred. At doses from 250 mg/kg bw/d there was a dose-related reduction in bodyweight gain and a significant bodyweight loss during the treatment period. The findings were associated with reduced food consumption. Pregnancy status was not affected by treatment, nor were the numbers of corpora lutea, implantation sites, pre- and post-implantation losses and live foetuses per litter. There were no dead or aborted foetuses in any group, and foetal weights were unaffected by treatment. Foetal external and visceral examinations revealed no treatment-related or toxicologically significant findings, and the same was found for skeletal malformations and variations. The incidence of skeletal anomalies (consisting mainly of fused or asymmetric sternebrae) was however slightly increased in the two higher dose groups, and occasionally slightly outside the HCD range. Statistical significance was reached only for the occurrence of fused sternebrae-3 and -4 in the high dose group. Additional analysis of the individual foetal data showed that severe sternebrae fusions were not reported and that there was no evidence of a treatment-related effect of fusion of all segments of the sternum (sternebrae 1 to 5). There were some differences from controls in the foetal incidence of partially fused and asymmetrically shaped sternebrae at the two higher doses, but these represent small deviations from normal sternal development, and are common variations in this rabbit strain (as shown by HCD) that have no effect on survival and do not persist postnatally. Given further that the findings occurred only in the presence of marked maternal toxicity, the DS concluded that, overall, this minor developmental change does not support classification.

Adverse effects on or via lactation

In the 2-generation study in rats, a treatment-related reduction in pup body weight was observed during the lactation periods of each generation. No data are available on the quality or quantity of the milk produced by the dams in this study, nor was the milk analysed for presence of trifloxystrobin or its metabolites. Studies in lactating ruminants, however, showed that trifloxystrobin is not excreted in the milk to any appreciable extent in cows or goats. This is not expected to be different in rats, and the DS considered it more likely that the reduced body weights in pups were partly due to the consumption of treated diet from PND12 (or avoidance of treated diet, on the basis of palatability). Reductions in body weight prior to PND12 were likely to be a non-specific secondary effect of maternal toxicity, as evidenced by reduced body weights, food consumption and slight kidney/liver toxicity in the mothers. Given that there is no clear evidence of an adverse effect due to transfer in the milk, or an adverse effect on the quality of the milk, the DS concluded that trifloxystrobin does not meet the criteria for classification for effects on or via lactation.

Comments received during public consultation

Two member state competent authorities (MSCAs) supported the 'no classification' proposal for effects on sexual function and fertility. One MSCA also supported the 'no classification' proposal for developmental toxicity, but two other MSCAs considered a classification in category 2 warranted, one on the basis of the sternebrae findings in rabbits, the other additionally also on the enlarged thymus findings in rats. The latter MSCA requested further details on the HCD for both findings. The DS, in response, provided the requested details, but remained of the opinion that enlarged thymus is a relatively frequent finding in the rat strain studied and that it is a variation without postnatal consequences, as shown by the absence of thymus lesions in the 2-generation study conducted with trifloxystrobin in the same rat strain and in the same laboratory. Regarding the sternebrae findings in rabbits, the DS pointed to the additional information on the severity of the sternebrae fusions as provided by IND during the public consultation (see Additional key elements). This additional information concerned a re-evaluation of the sternebrae findings based on photographs recently taken from skeletal preparations from the original rabbit developmental toxicity study. Most sternebrae findings could be considered small deviations from the normal situation at the end of the gestation period without long-term postnatal consequences. According to the DS these can thus be downgraded to variations and would not support classification for developmental toxicity, particularly when the maternal toxicity (as evidenced by severe weight loss, resulting in a reduced body weight gain from GD7-20 of -130 % and -238 % of control at 250 and 500 mg/kg bw/d, respectively) and significantly reduced food consumption (by more than 50 %) during the treatment period) is taken into account.

The 'no classification' proposal for effects on or via lactation was supported by three MSCAs, agreeing with the DS that there is insufficient evidence directly linking the pup weight effects to lactation and that the criteria for classification are thus not considered fulfilled. One other MSCA however considered classification with Lact.; H362 warranted.

Additional key elements

During the public consultation, IND submitted a re-evaluation of the sternebrae fusions in the rabbit developmental toxicity study, to better inform on the severity and the toxicological

significance of the findings. Photographs were made of archived alizarin red S stained skeletal preparations of all (but one) foetuses previously recorded as having any degree of sternebrae fusion, and these were re-analysed. A distinction was made between 1) abnormal sternum (defined as "a complex combination of fusions and other morphological changes which could possibly have an impact upon subsequent development", considered to be a malformation), 2) sternebrae fusion (either fused ossification, partially fused ossification or bridge of ossification, considered to be minor anomalies/variations), and 3) other sternebrae observations (slightly asymmetrically ossified sternebrae, slightly misaligned sternebrae or offset hemisternebrae). The results are presented in the table below.

***Table**: Re-evaluation of affected sternebrae in rabbit foetuses. If a foetus was having more than one degree of severity of fusion between sternebrae, the more severe class of fusion was included in the table.*

	Dose (mg/kg bw/d)				
	0	10	50	250	500
No. foetuses (litters)	116 (19)	130 (18)	90 (16)	97 (17)	97 (18)
No. re-evaluated foetuses (litters) with sternebrae fusions	5 (5)	4 (4)	4 (4)	11 (8)$^{+}$	14 (6)$^{+}$
Severity of fusion					
Abnormal sternum	1 (1)	1 (1)	1 (1)	1 (1)	3 (3)
Sternebrae 4-5 fused	-	-	-	3 (3)	2 (2)
Partially fused sternebrae	3 (3)	2 (2)	1 (1)*	3 (3)	4 (4)
Bridge of ossification	1 (1)	1 (1)	2 (2)	4 (4)	5 (5)
Other sternebrae observations (excluding abnormal sterna)					
Fusion eccentric not midline	-	-	-	1 (1)	1 (1)
Slightly misshapen	-	-	-	1 (1)	2 (2)
Slightly misaligned	1 (1)	-	-	2 (2)	2 (2)
Slightly asymmetrically ossified	1 (1)	-	1 (1)	1 (1)	-
Slightly shortened/thickened	-	-	-	-	1 (1)
5-6 incompletely ossified	2 (2)	1 (1)	3 (3)	10 (8)	9 (4)

$^{+}$ one litter may contain more than one affected foetus
* sternum not available for re-evaluation – data taken from original study report

At 0, 10 and 50 mg/kg bw/d, one sternum in each group was found to have major fusions and associated sternebrae changes and was classified as abnormal. In addition 4, 3 and 3 foetuses from 4, 3 and 3 litters had lesser degrees of sternebrae fusion and the changes were considered to be minor anomalies/variations. At 250 mg/kg bw/d, also 1 foetus was found to have an abnormal sternum. The incidence of foetuses/litters with minor sternebrae anomalies was, however, slightly increased, with 10 foetuses from 8 litters affected. At 500 mg/kg bw/d, of the 14 sterna from 6 litters examined, 3 sterna from 3 different litters were classified as abnormal. The remaining 11 foetuses from 5 litters had minor sternebrae anomalies/variations. One of these litters (dam number 93) contained 1 foetus with an abnormal sternum and 4 foetuses with minor sternebrae changes.

It is reported that aside from one foetus at 250 mg/kg bw/d, none of the foetuses with sternebrae fusions had abnormalities of the rib cage and/or vertebral column that might have contributed to or arisen from the sternebrae changes.

Overall it was concluded in the re-evaluation report that there were slight increases in minor sternebrae anomalies/variations at 250 and 500 mg/kg bw/d, and at 500 mg/kg bw/d two additional instances of abnormal sternum. On a weight of evidence basis, however, no classification was considered warranted for these findings given that:

- there were no other skeletal and visceral adverse changes and no increase in embryo-foetal
- deaths;
- the sternebrae fusions only occurred in the presence of considerable maternal toxicity, as evidenced by body weight loss during the first half of the treatment period and markedly reduced food consumption throughout the treatment period;
- with the exception of abnormal sternum, the other sternebrae fusions represent small deviations from the normal situation. They may indicate a slight advancement in ossification of the sternum, but premature fusions are not thought to have long-term post-natal consequences;
- there was no indication on GD29 that the integrity of the rib cage and vertebral column had been affected by the abnormal sterna;
- no sternebrae abnormalities were seen in the rat developmental toxicity study despite the highest dose level being twice that used in the rabbit developmental toxicity study.

Assessment and comparison with the classification criteria

Sexual function and fertility

No treatment-related effects on fertility parameters were observed in the 1- and 2-generation studies in rats. The trifloxystrobin doses tested in these studies (up to 2 000 ppm and 1 500 ppm, respectively), however, resulted in only limited parental toxicity (no evidence of treatment-related mortality or clinical signs, only slightly reduced body weight at several time points throughout treatment, in association with reduced food consumption). Higher doses were not given in view of the pup toxicity seen (retarded weight development during lactation) at 2 000 ppm in the 1-generation study, as well as the (slight) parental toxicity in that study. RAC notes that this is not a proper argument and questions whether the top dose used in the 2-generation study (1 500 ppm; equal to 73.1-126.7 mg/kg bw/d for males and 98.0-242.0 mg/kg bw/d for females) was optimal. This raises the issue of whether the fertility endpoint was fully investigated or if the 2-generation study was truly OECD 416 test guideline compliant with regard to the selection criteria for determining the highest dose.

In addition to the 1- and 2-generation studies, the CLH report includes short summaries of a 90-d study (0/100/500/2 000 ppm in males and 0/100/500/2 000/8 000 ppm in females) and a 2-y study (0/50/250/750/1 500 ppm) in rats. In these studies no adverse effects on the reproductive organs were observed. It is noted that the DS considered the higher dose levels in these studies to have exceeded the maximum tolerated dose (MTD) (given mortality (90-d study) and large reductions in body weight gain). NOAELs were established at 500 and 250 ppm for the 90-d and 2-y study, respectively, as assessed by EFSA in their pesticides peer review report on trifloxystrobin. RAC agrees that 8 000 ppm for females in the 90-d study (equal to 618 mg/kg bw/d) exceeded the MTD with 20 % of the animals dying. However, 2 000 ppm tested in the 90-d study and 1 500 ppm in the 2-y study were tolerated without excessive toxicity.

On the basis of the data available, no classification for effects on fertility and sexual function is warranted. RAC, however, notes that the available 2-generation study may not fully inform on this endpoint.

Developmental toxicity

Rats

In a guideline-compliant rat developmental toxicity study (0, 10, 100, 1 000 mg /kg bw/d on GD6-15), the only possibly treatment-related effect observed was a statistically significant increase in the incidence of foetuses with enlarged thymus at the top dose group. The incidence was slightly outside the HCD range, both on a foetal and on a litter base (see table below). In the report presenting the laboratory HCD, it is stated that the in-life phase of the studies was within 3 years of the reference study (study number 943042; not included in the database). IND has confirmed that the reference study with number 943042 is indeed the rat study with trifloxystrobin and that this study was conducted in 1994.

***Table**: Incidences of enlarged thymus in the rat prenatal developmental toxicity study*

	Dose (mg/kg bw/d)				
	0	10	100	1 000	HCD# (mean; range)
Enlarged thymus					
No. foetuses affected/ total no. examined (foetal incidence)	3/149 (2.0 %)	3/135 (2.2 %)	3/139 (2.2 %)	11/146 (7.5 %)*	13/4 793 [anomaly] (0.3 %; 0.0-1.4 %) 32/4 793 [variation] (0.7 %; 0.0-6.0 %)
No. litters affected/ total no. examined (litter incidence)	3/23 13.0 %)	1/22 (4.5 %)	3/20 (15.0 %)	7/22 (31.8 %)	13/725 [anomaly] (1.8 %; 0.0-9.5 %) 29/725 [variation] (4.0 %; 0.0-29.2 %)

HCD: historical control data from 22 gavage studies with in total 31 control groups; studies conducted in 1988-1994, in same laboratory and rat strain. Database does not include study number 943042 (the trifloxystrobin study). Finding was first categorized as an anomaly but from mid-1992 as a variation, because in these later studies it was seen more often.
* Statistically significant ($p < 0.05$)

From the table above, it can be seen that the number of foetuses/litters with enlarged thymus at the low and mid dose groups was not different from that in the concurrent control group, whereas at the high dose group it was slightly higher and slightly outside the HCD ranges from the mid-1992 to 1994 studies. It is noted though that in all groups the incidences exceeded the mean incidence of the HCD.

RAC notes that the degree of enlargement is not given, making it difficult to discriminate between an anomaly and a variation. According to the DS, "enlarged thymus" is a descriptive finding of the technician/study director not based on exact size measurements. Furthermore, the laboratory where the study was conducted downgraded the finding from an anomaly to a variation ("Relatively frequent, transient structural deviation from normal development that is considered not to have any detrimental effect on fetal survival, development or function. Variations occur frequently in control fetuses."), given that from mid-1992 it occurred more regularly in the rat strain.

Considering that enlarged thymus is a relatively frequent finding in the rat strain studied, that the increase in incidence was relatively small and occurred only at the limit dose of 1 000 mg/kg bw/d which was maternally toxic (32 % lower net weight change over GD6-21 as compared to controls), that no thymus lesions were observed in the 2-generation study and that thymus was not a target organ in other repeated dose toxicity studies, RAC concludes that it does not provide sufficient evidence for classification.

Rabbits

In a guideline-compliant rabbit developmental toxicity study (0, 10, 50, 250, 500 mg/kg bw/d of trifloxystrobin on GD7-19), the only treatment-related finding was a slightly increased incidence of skeletal anomalies in foetuses in the two higher dose groups. These consisted mostly of fused sternebrae and asymmetrically shaped sternebrae, with the incidences for some sternal findings slightly exceeding the HCD range (see table below). The laboratory HCD were from studies for which the in-life phase was reported to be within 3 years of the reference study (study number 943043; included in the database). IND has confirmed that the reference study with number 943043 is indeed the rabbit study with trifloxystrobin and that this study was conducted in 1994.

As can be seen from the table below, most foetal and litter incidences found for the skeletal anomalies in the treated groups exceeded the mean incidences of the HCD. Only occasionally though they (slightly) exceeded the HCD range. The increases in incidence were only small and mostly without dose response relation. There was only one statistically significant finding, for the occurrence of fused sternebrae 3-4 in the highest dose group.

***Table**: Incidences of skeletal anomalies in the rabbit prenatal developmental toxicity study*

		Dose (mg/kg bw/d)					HCD# (mean; range)
		0	10	50	250	500	
Skeletal anomalies							
Sternebra 1, Fused 1-2	No. foetuses affected	1/116 (0.9 %)	1/130 (0.8 %)	1/90 (1.1 %)	2/97 (2.1 %)	1/97 (1.0 %)	8/2 562 (0.3 %; 0.0 %-2.5 %)
	No. litters affected	1/19 (5.3 %)	1/18 (5.6 %)	1/16 (6.3 %)	2/17 (**11.8 %**)	1/18 (5.6 %)	8/455 (1.8 %; 0.0 %-10.5 %)
Sternebra 1, asymmetrically shaped	No. foetuses affected	0/116 (0.0 %)	1/130 (0.8 %)	1/90 (1.1 %)	2/97 (2.1 %)	3/97 (**3.1 %**)	10/2 562 (0.4 %; 0.0 %-2.3 %)
	No. litters affected	0/19 (0.0 %)	1/18 (5.6 %)	1/16 (6.3 %)	1/17 (5.9 %)	1/18 (5.6 %)	10/455 (2.2 %; 0.0 %-13.3 %)
Sternebra 2, Fused 2-3	No. foetuses affected	1/116 (0.9 %)	1/130 (0.8 %)	1/90 (1.1 %)	4/97 (4.1 %)	4/97 (4.1 %)	25/2 562 (1.0 %; 0.0 %-5.7 %)
	No. litters affected	1/19 (5.3 %)	1/18 (5.6 %)	1/16 (6.3 %)	4/17 (**23.5 %**)	4/18 (**22.2 %**)	23/455 (5.1 %; 0.0 %-20.0 %)
Sternebra 2, asymmetrically shaped	No. foetuses affected	0/116 (0.0 %)	1/130 (0.8 %)	1/90 (1.1 %)	2/97 (2.1 %)	4/97 (4.1 %)	16/2 562 (0.6 %; 0.0 %-4.1 %)
	No. litters affected	0/19 (0.0 %)	1/18 (5.6 %)	1/16 (6.3 %)	2/17 (11.8 %)	3/18 (**16.7 %**)	15/455 (3.3 %; 0.0 %-13.3 %)
Sternebra 3, Fused 3-4	No. foetuses affected	2/116 (1.7 %)	2/130 (1.5 %)	1/90 (1.1 %)	5/97 (5.2 %)	10/97* (**10.3 %**)	68/2 562 (2.7 %; 0.0 %-9.2 %)
	No. litters affected	2/19 (10.5 %)	1/18 (5.6 %)	1/16 (6.3 %)	4/17 (23.5 %)	6/18 (33.3 %)	57/455 (12.5 %; 0.0 %-33.3 %)
Sternebra 3, asymmetrically shaped	No. foetuses affected	0/116 (0 %)	1/130 (0.8 %)	0/90 (0 %)	2/97 (2.1 %)	3/97 (**3.1 %**)	14/2 562 (0.5 %; 0.0 %-2.7 %)
	No. litters affected	0/19 (0 %)	1/18 (5.6 %)	0/16 (0 %)	2/17 (**11.8 %**)	3/18 (**16.7 %**)	13/455 (2.9 %; 0.0 %-10.5 %)
Sternebra 4, Fused 4-5	No. foetuses affected	4/116 (3.4 %)	2/130 (1.5 %)	4/90 (4.4 %)	7/97 (7.2 %)	8/97 (**8.2 %**)	68/2 562 (2.7 %; 0.0 %-8.0 %)
	No. litters affected	4/19 (21.1 %)	2/18 (11.1 %)	4/16 (25.0 %)	6/17 (**35.3 %**)	6/18 (**33.3 %**)	57/455 (12.5 %; 0.0 %-29.4 %)
Sternebra 4, asymmetrically shaped	No. foetuses affected	0/116 (0 %)	1/130 (0.8 %)	0/90 (0 %)	4/97 (**4.1 %**)	2/97 (2.1 %)	23/2 562 (0.9 %; 0.0 %-3.2 %)
	No. litters affected	0/19 (0 %)	1/18 (5.6 %)	0/16 (0 %)	4/17 (**23.5 %**)	2/18 (11.1 %)	22/455 (4.8 %; 0.0 %-17.6 %)
Sternebra 5, asymmetrically shaped	No. foetuses affected	0/116 (0 %)	0/130 (0 %)	0/90 (0 %)	0/97 (0 %)	1/97 (1.0 %)	18/2 562 (0.7 %; 0.0 %-2.7 %)
	No. litters affected	0/19 (0 %)	0/18 (0 %)	0/16 (0 %)	0/17 (0 %)	1/18 (5.6 %)	18/455 (4.0 %; 0.0 %-17.6 %)
Sternebra 6, asymmetrically shaped	No. foetuses affected	0/116 (0 %)	0/130 (0 %)	1/90 (1.1 %)	1/97 (1.0 %)	0/97 (0 %)	7/2 562 (0.3 %; 0.0 %-2.3 %)
	No. litters affected	0/19 (0 %)	0/18 (0 %)	1/16 (6.3 %)	1/17 (5.9 %)	0/18 (0 %)	7/455 (1.5 %; 0.0 %-13.3 %)

HCD: historical control data from 20 gavage studies with in total 24 control groups; studies conducted in 1989-1995, in same laboratory and rabbit strain. Database includes study number 943043 (the trifloxystrobin study). Values exceeding HCD range are **in bold**.
* Statistically significant ($p < 0.05$)

In the CLH report, the sternal findings were also presented on individual foetus basis (see table below), on the basis of which the DS concluded that severe sternebrae fusion, alignment of ribs with the sternebrae or abnormal curvature of the sternum, which could possibly impair postnatal development resulting in a shortened rib cage with consequently impairment of further pup development, did not occur. There was a slight increase in foetuses with fused sternebrae (2-1-1-3-3 at 0-10-50-250-500 mg/kg bw/d) and partially fused sternebrae (3-3-3-8-11 at 0-10-50-250-500 mg/kg bw/d) in the two highest dose groups, but the more severe finding of fusion of all segments of the sternum (sternebrae 1 to 5) was not increased (1-0-1-1-1 at 0-10-50-250-500 mg/kg bw/d).

***Table**: Details of sternal findings in the rabbit prenatal developmental toxicity study*

		Dose (mg/kg bw/d)				
		0	10	50	250	500
No. foetuses examined		116	130	90	97	97
No. litters examined		19	18	16	17	18
Sternebra(e)		***identification number dam/identification number foetus***				
Sternebra 1 and 2	Fused	7/8	25/6		63/7	
	Partially fused			55/1	65/6	93/1
Sternebra 2 and 3	Fused				63/7	93/1
	Partially fused	7/8	25/6	55/1	65/4, 66/7, 74/4	84/1, 86/3, 87/5
Sternebra 3 and 4	Fused	7/8	25/6	55/1		93/5
	Partially fused	11/5	25/3		63/1, 63/7, 66/7, 67/7, 74/4	82/3, 84/2, 85/5, 86/3, 87/5, 93/1, 93/2, 93/6, 93/8
Sternebra 4 and 5	Fused	7/8, 9/1		55/1	66/7, 70/4	82/3, 93/1
	Partially fused	10/1, 17/2	32/7, 36/1	39/7, 48/2, 51/9	62/3, 63/7, 71/1, 71/7, 74/4	84/7, 85/4, 86/3, 86/7, 87/5, 93/6
Asymmetrically shaped			25/6	55/1	62/3, 63/1, 63/7, 63/8, 72/4, 74/4	86/3, 87/5, 93/1, 93/5, 93/6
No. of foetuses with						
Fused sternebra(e)		2	1	1	3	3
Partially fused sternebra(e)		3	3	3	8	11
Fusion of sternebrae 1-5		1	0	1	1	1

According to the DS, fused and asymmetric sternebrae are grey zone anomalies that, depending on their severity, can be upgraded (to malformations) or downgraded (to variations). The table above, however, gives no indication of the severity of the fusions, and therefore IND presented a re-evaluation of the sternebrae fusions during the public consultation, to better inform on the severity and toxicological significance of the findings (see the Background Document). The re-evaluation shows that at 250 and 500 mg/kg bw/d there were slight increases in the number of foetuses/litters with fused sternebrae 4-5, partially fused sternebrae and bridges of ossification. At 500 mg/kg bw/d there was additionally a small increase in the number of foetuses/litters with an abnormal sternum (two additional cases as

compared to the other dose/control groups). However, except for one foetus at 250 mg/kg bw/d (number 74/4), none of the foetuses with sternebrae fusions appeared to have abnormalities of the rib cage and/or vertebral column. Hence, apparently postnatal and further pup development was not impaired in the foetuses with skeletal anomalies.

RAC notes that the sternal findings only occurred at doses where there was considerable maternal toxicity, even though this toxicity may not be responsible for the sternal findings. At 250 and 500 mg/kg bw/d, the dams had significant body weight losses compared to controls during the treatment period (-83 and -152 g over GD7-20, respectively, as compared to a body weight gain of 64 g for controls), associated with a decreased food consumption during these days, in particular during GD7-12 (reduction by above 50 % at 250 and 500 mg/kg bw/d). Since trifloxystrobin was administered by gavage, palatability could not have been the reason of the decreased food consumption.

RAC further notes that a higher dose of trifloxystrobin in rats (1 000 mg/kg bw/d) did not result in increases in skeletal anomalies. There was a statistically significant increase seen in the occurrence of asymmetrically shaped sternebra 1, but only in foetuses of the low dose group (10 mg/kg bw/d), not in foetuses of the mid and high dose groups (100 and 1 000 mg/kg bw/d). Incidences of all other asymmetric and fused/partially fused findings of the sternebrae also showed no dose relation.

RAC finally notes that in rabbits, there were no other external, visceral or skeletal adverse findings and no increase in post-implantation loss that could have masked an increase in foetal abnormalities.

In conclusion, RAC agrees with the DS that most fused/partially fused and asymmetric sternal findings seen at 250 and 500 mg/kg bw/d are likely to represent small deviations from normal sternal development that have no effect on survival and do not persist postnatally. Fusion of all segments of the sternum (sternebrae 1 to 5), which is considered a more severe finding, was not increased by treatment. Another more severe finding, abnormal sternum, was increased at 500 mg/kg bw/d and could possibly warrant classification. However, as the increase was only small (two additional instances) and on GD29, the integrity of the rib cage and vertebral column did not appear to have been affected by the abnormal sterna, RAC considers this not to present sufficient evidence for classification, taking into account all the evidence as discussed in the paragraphs above.

Adverse effects on or via lactation

In the 2-generation study in rats, dietary treatment with 750 and 1 500 ppm trifloxystrobin resulted in a treatment-related reduction in male and female pup body weight during the lactation periods of each generation. At LD0, the mean weight of pups in all treated groups was equivalent to that of the controls. But from LD7 onwards it was statistically significantly decreased, see table below. A similar effect was seen in the 1-generation range-finding study at 2 000 ppm. For pups at 1 500 ppm, mean values for eye opening during the lactation period were statistically significantly delayed by 0.7 (F1a), 0.6 (F1b) or 0.7 (F2) days; this may have been secondary to the reduced body weight development.

***Table**: Body weight development (% of controls) in F1a, F1b and F2 pups (males and females combined) in the rat 2-generation study*

	Dose (ppm)		
	50	750	1 500
LD0			
F1a	100	100	102
F1b	105	102	107
F2	102	100	102
LD4 after reduction			
F1a	103	101	93
F1b	104	99	93
F2	103	97	93
LD7			
F1a	101	97	85**
F1b	103	94	84**
F2	101	91**	84**
LD14			
F1a	100	94*	79**
F1b	101	91**	78**
F2	101	89**	78**
LD21			
F1a	99	91**	72**
F1b	100	89**	73**
F2	100	86**	72**

* Statistically significant ($p < 0.05$)
** Statistically significant ($p < 0.01$)

As the effect at 750 and 1 500 ppm is statistically significant, dose-related and consistent over the sexes and generations, with effect sizes up to 14 and 28 %, respectively, it may be considered adverse. It is therefore important to consider whether it qualifies for classification for developmental toxicity or for effects on or via lactation. When assessing the effect, the following observations are of note:

1. The F0/F1 parental generations also show a reduced body weight relative to controls with trifloxystrobin treatment, in the same order of magnitude.
2. It does not seem to be a specific developmental effect, as there was no effect on pup body weight at birth. There was also no *in utero* effect on mean foetal body weight at (gavage) doses up to 1 000 mg/kg bw/d in the rat developmental toxicity study.
3. There was no loss in body weight amongst pups. All pups continued to thrive throughout PND 1-21.
4. Although the 750/1 500 ppm F1 males and females selected to breed the F2 generation still had lower body weights when pre-mating started, there were no adverse effects on fertility (e.g. fertility index, duration of gestation, gestation index and litter size were not affected, and were not different from the F0 generation).
5. As the only developmental delay reported was a slight delay in eye opening in pups at 1 500 ppm, the onset of puberty/sexual maturation may be considered as not having been affected.
6. The survival of pups at 750 and 1 500 ppm was not affected, as viability and lactation indices were both in the range of 96.6-99.4 %.

From this it seems that classification for developmental toxicity is not warranted, as the significant adverse effect on rat F1 and F2 post-natal pup bodyweight does not appear to be a specific developmental effect and was without significant impact on later maturation and fertility.

For classification for effects on or via lactation, the CLP criteria require:

a) Human evidence indicating a hazard to babies during the lactation period

No such data from humans are available for trifloxystrobin.

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

There is no indication of behavioural changes in dams that could have affected weight gain development of the pups, as dams of all dose groups successfully reared their litters to weaning on day 21 post-partum. No information is available on the quantity or quality of the milk produced by the dams, nor was the rat milk analysed for the presence of trifloxystrobin or metabolites. The CLH report refers to three studies in ruminants (two in goats, one in cows) in which trifloxystrobin and its metabolites were not excreted in milk to an appreciable extent (in goats 0.06-0.08 % of the totally administered dose; in cows below the limit of quantification of 0.01 mg/kg). Given these results, the DS considered it highly unlikely that trifloxystrobin or its metabolites would be transferred into the milk of rats. RAC, however, notes that the doses in the ruminant studies were rather low (goats were administered 4.13-4.24 mg/kg bw trifloxystrobin for 4 days, cows received 0.065, 0.193 or 0.635 mg/kg bw/d trifloxystrobin for 28-30 days).

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

According to the DS, the available toxicokinetic data do not suggest likely transfer to milk, given rapid metabolism of trifloxystrobin to more polar, water-soluble molecules that are excreted primarily via urine and bile. In support, the studies with goats and cows showed no appreciable transfer to milk. Further, the unchanged parent compound was the major component in milk and fat of goats, for which the metabolic pathway of trifloxystrobin was similar to that in rats. Overall, the DS considered it highly unlikely that trifloxystrobin or its metabolites would be transferred into the milk of rats. RAC, however, notes that the log P_{OW} of 4.5 indicates lipophilicity of the substance and thus some potential for transfer to milk. RAC further notes the low dosing in the studies with goats and cows.

In absence of data on transfer into milk or on quality of the milk, the available data unfortunately do not allow directly linking the effect to lactation. Other possibilities include a direct effect of pups consuming treated diet (or avoiding it, because of palatability reasons), as solid food intake starts from around LD14. Whereas this may contribute to the reduced body weight development during the later phase of the lactation period, it does not explain the effect seen at LD7 when the pups are still mostly breast-fed only. Another possibility is that the retarded body weight development is a secondary effect of maternal toxicity (i.e., reduced food consumption and lower body weights during lactation in the parental females). However, this maternal toxicity was also seen during gestation, where it did not result in an effect on body weight of the pups at birth (LD0). Thus, discarding these possibilities, the most plausible explanation is that the effect is caused through the milk, given the log P_{ow} and the fact that it partly occurred during a time period where milk is the only nutrition source for pups.

With consistency seen over two generations, two studies and two sexes, **RAC considers classification for effects on or via lactation (Lact.; H362) justified.**

10.11 Specific target organ toxicity-single exposure

Not considered in this report.

10.12 Specific target organ toxicity-repeated exposure

10.12.1 Summary of available data

This end point is not evaluated in this report. However, summaries of the 28-day, 90-day and 2-year chronic toxicity studies in rats are provided below as supplementary information for the evaluation of reproductive toxicity hazard classification (section 10.10).

Table 19: Summary table of animal studies on repeated-dose toxicity of trifloxystrobin

↑ / ↓ = increased/decreased compared with control. MTD = Maximum Tolerated Dose.

Method Guideline, Deviation(s) from the guideline (if any)	Species, strain, sex, no/group	Dose levels duration of exposure	Results
Sub-acute 28-day oral study (dietary) Non-guideline study Non-GLP Anonymous (1994b), M-040074-01-1 Guidance values: Cat 1: C ≤ 30 Cat 2: 30 < C ≤ 300 (calculated using Haber's rule)	Rat, Sprague-Dawley 5/sex/group	Trifloxystrobin (purity: 96.2 %) 0, 200, 1000, 4000, 12000 ppm Equivalent to: Males: 0, 16.51, 84.35, 337.2, 1074 mg/kg bw/day Females: 0, 16.37, 84.06, 327.0, 1005 mg/kg bw/day	<u>200 ppm (16.51/16.37 mg/kg bw/d)</u> No adverse effects <u>1000 ppm (84.35/84.06 mg/kg bw/d)</u> Decreased body weight gain (↓13 %, males) Slightly decreased food consumption (males) <u>4000 ppm (337.2/327.0 mg/kg bw/d)</u> Soft faeces & diarrhoea Decreased body weight gain(↓22 %, males) Slightly decreased food consumption(males) Slightly increased plasma albumin & cholesterol levels (both sexes), glucose (males) & urea levels (females) Increased relative liver weight (↑13 %, males) <u>12000 ppm (1074/1005 mg/kg bw/d)</u> Soft faeces & diarrhoea Decreased body weight gain (↓34 %, males; ↓27 %, females) Slightly decreased food consumption (both sexes) Slightly increased plasma albumin, cholesterol levels, glucose & urea levels (both sexes) Increased relative liver weight (↑31 % and 15 % in males and females, respectively), relative kidney weight (both sexes) and relative adrenal weight (males only)
Sub-chronic 90-day oral study (dietary) OECD 408 GLP Trifloxystrobin (purity: 96.2 %) Anonymous (1997d), M-040135-01-1 Guidance values: Cat 1: C ≤ 10 Cat 2: 10 < C ≤ 100	Rat, Sprague-Dawley 15/sex/group Control and top dose groups: Additional 10/sex for 4 week recovery period	Both sexes: 0, 100, 500, 2000 ppm Females only: 8000 ppm Equivalent to: Males: 0, 6.4, 30.6, 127 mg/kg bw/day Females: 0, 6.8,	<u>100 ppm (6.4/6.8 mg/kg bw/d)</u> No adverse effects <u>500 ppm (30.6/32.8 mg/kg bw/d)</u> Decreased mean terminal body weight gain (↓9 %, males) and food consumption (6 – 10 %, males) Increased relative liver weights (↑13 %, males), partly reversible after recovery <u>2000 ppm (127/133 mg/kg bw/d)</u> **MTD exceeded in males** 1/25 females found dead (day 16) and 1/25 males sacrificed in moribund condition (day 35), with histopathology findings in numerous organs (atrophy of the parenchyma); single animals with

Method Guideline, Deviation(s) from the guideline (if any)	Species, strain, sex, no/group	Dose levels duration of exposure	Results
		32.8, 133, 618 mg/kg bw/day	reduced bodyweight Decreased mean terminal body weight gain (↓20 % in males; ↓17 % in females) and food consumption (↓6 – 10 %, both sexes) Slightly decreased water consumption (males, during first 4 weeks) Slightly decreased plasma globulin & total protein (both sexes) Slightly increased cholesterol levels (males), partly reversible during recovery Increased relative liver weights (↑22 %, males) and kidney weights (↑12 %,males) Organ weight changes partly reversible after recovery Hepatocellular hypertrophy (minimal, 5/10 males), pancreas atrophy (2/10 males and 1/9 females in main group; 2/10 males in recovery group) <u>8000 ppm (females only, 618 mg/kg bw/d)</u> **MTD exceeded** 1/25 females found dead (day 28) and 4/25 females sacrificed in moribund condition (days 30-34), with histopathology findings in liver, kidney (acute tubular lesions) and atrophy of the parenchyma in several organs; single animals with reduced bodyweight Transient piloerection (week 1, 25/25 females) Soft faeces (week 1, 25/25 females) Decreased mean terminal body weight gain (↓40 %),food consumption (6 – 10 %) and overall water consumption (↓11 %, reversible within recovery period) Slightly increased RBCs, Hb, Hct and tendency to eosinophilia, reversible within recovery period Slightly decreased plasma globulin & total protein Increased glucose, urea and potassium levels, partly reversible during recovery slightly acidic urine Increased relative liver weights (↑39 % relative) and kidney weights (↑14 % relative) Organ weight changes partly reversible after recovery Small thymus (3/13, 1/8 after recovery) Hepatocellular hypertrophy (minimal, 7/8), pancreas atrophy (7/8), salivary gland atrophy (1/8) NOAEL: 30.6 mg/kg bw/day (EFSA conclusion on pesticides peer review, 2017-09-14)
Chronic 2-year oral study (dietary) OECD 453 GLP Trifloxystrobin (purity: 96.4 %) Anonymous (1998d), M-040512-02-1	Rat, Sprague-Dawley 80/sex/group	0, 50, 250, 750, 1500 ppm Equivalent to: Males: 0, 1.95, 9.81, 29.7	<u>*Neoplastic findings*</u> Not relevant for this kind of report <u>*Non-neoplastic findings*</u> <u>50 ppm (1.95/2.22 mg/kg bw/d)</u> No adverse effects <u>250 ppm (9.81/11.4 mg/kg bw/d)</u>

Method Guideline, Deviation(s) from the guideline (if any)	Species, strain, sex, no/group	Dose levels duration of exposure	Results
Guidance values: Cat 1: C ≤ 1.25 Cat 2: 1.25 < C ≤ 12.5 (calculated using Haber's rule)		62.2 mg/kg bw/day Females: 0, 2.22, 11.4, 34.5, 72.8 mg/kg bw/day	Decreased body weight gain (up to ↓7.4 % in females) and food consumption (↓4 % in females) <u>750 ppm (29.7/34.5 mg/kg bw/d)</u> Decreased body weight gain (up to ↓5.3 % and ↓10.8 % in males and females, respectively) and food consumption (↓4 % in females) Increased relative liver weights (week 53; ↑11 % in males) Decreased incidence of large pituitary gland (females) Decreased incidence of fatty change in liver and fatty atrophy in pancreas, both in females Hepatocellular hypertrophy (1/50 females) <u>1500 ppm (62.2/72.8 mg/kg bw/d)</u> Increased incidence of diarrhoea (males, towards end of study) Decreased body weight gain (up to ↓16.3 % and ↓26.2 % in males and females, respectively), body weight (1-year sacrifice females: ↓19%, terminal females: ↓16%), food consumption (↓4 % in males; ↓8 % in females) and water consumption (females) Increased relative liver weights (week 53: ↑10 % in males, ↑24 % relative in females; terminal sacrifice: 9 % relative in females), kidney weights (week 53: ↑20 % in females; terminal sacrifice: ↑12 %in females), testes weights (terminal sacrifice: ↑22 %) Decreased incidence of large pituitary gland (both sexes), incidence of fatty change in liver and fatty atrophy in pancreas Hepatocellular hypertrophy (2/50 females) NOAEL: 10 mg/kg bw/day (EFSA conclusion on pesticides peer review, 2017-09-14)

<u>28-day study in the rat</u>

This study was conducted as a range-fnding study and did not include histopathological examination. The main findings were significant dose related lower body weight gain at 1000 ppm and above, and increases in relative weights of liver (≥4000 ppm), kidneys and adrenals (12000 ppm).

<u>90-day study in the rat</u>

In the 90-day study the top doses in males (2000 ppm) and in females (8000 ppm) were considered to have exceeded the MTD based on mortality and the large reduction in body weight gain compared with that of controls. Some recovery in body weight was observed during the 4-week recovery period but terminal body weights remained lower than controls. Food intake was also reduced but was higher than that of controls during the recovery period. Minimal to slight changes in a number of clinical pathology parameters were of minor toxicological significance and may have been associated with overt general toxicity.

Statistically significant increases in relative liver and kidney weights were noted in males at 500 (liver only) and 2000 ppm and in females at 8000 ppm. Although absolute values for these organs were also mostly higher than control values there was no clear treatment relationship. Liver and kidney weight changes were partly

reversible after recovery. The increased liver weights were likely associated with an increased incidence of minimal hepatocellular hypertrophy at the top dose levels. Atrophy in a number of organs of decedent animals was probably associated with their moribund condition. A minimal to moderate atrophy of the pancreas was seen in most top dose group females and a minimal atrophy in one female dosed at 2000 ppm. A minimal atrophy of the pancreas was observed in two top dose group males.

There were no treatment related pathological findings or organ weight effects in reproductive organs.

Following the EU Pesticides Peer Review teleconference 144, the NOAELs were concluded to be 500 ppm for both males and females (30.6 and 32.8 mg/kg bw/d respectively) based on reduced body weight gain, food consumption and increased organ weight (liver and kidney) (EFSA conclusion on pesticides peer review, 2017-09-14).

2-year study in the rat

Animals received trifloxystrobin in the diet for periods of up to one year (interim sacrifice) or for up to 2 years (terminal sacrifice) for assessment of chronic toxicity and carciogenicity. The assessment of neoplastic findings is not included in this dossier since it is not relevant to the supplementary information being provided for evaluation of the reproductive toxicity hazard classification.

The main findings indicative of chronic toxicity included: reduced body weight gain in both sexes at ≥750 ppm and in females at 250 ppm; reduced food intake at 750 and 1500 ppm (top dose); increased relative liver and kidney weights at the interim and terminal sacrifices at 750 and/or 1500 ppm; slightly decreased incidences of fatty changes in the liver and fatty atrophy in the pancreas in females at ≥ 750 ppm (likely associated with reduced body weights) and a very low incidence of hepatocellular hypertrophy in females at ≥750 ppm (terminal sacrifice).

There were no treatment related findings in reproductive organs. Increased testes weights noted in the top dose group at terminal sacrifice were deemed to be a consequence of fluid contents in the albugineous tunica of some animals. However there were no related microscopic findings, therefore this finding was not considered toxicologically significant.

The chronic toxicity NOAEL was established at 250 ppm (10 mg/kg bw/day) based on decreased body weight and confirmed after consideration at the EU Pesticides Peer Review teleconference 144 (EFSA conclusion on pesticides peer review, 2017-09-14).

10.12.2 Summary of repeated dose toxicity data

After oral (dietary) administration of trifloxystrobin reduced body weight and body weight gain associated with lower food consumption was consistently observed in all rat repeated dose toxicity studies at doses of 750 ppm and above.

Liver effects comprised increased relative liver weights and minimal hepatocellular hypertrophy. In addition, in the 2-year study lower incidence of fatty changes in the liver, lower incidence of fatty atrophy in the pancreas and a very low incidence of hepatocellular hypertrophy was observed in females at doses ≥750 ppm. Increased relative kidney weights were noted at doses ≥1500 ppm. Minimal to moderate acute tubular lesion in the kidney occurred only in females at a dose exceeding the MTD.

In the 90-day study the top doses in males (2000 ppm) and in females (8000 ppm) were considered to have exceeded the MTD based on mortality and the large reduction in body weight gain compared with that of controls. Atrophy in a number of organs of decedent animals was probably associated with their moribund condition.

There were no treatment related pathological findings or organ weight effects in reproductive organs.

10.13 Aspiration hazard

Not considered in this report.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Trifloxystrobin was originally included in Annex l of Council Directive 91/414/EEC on 1 October 2003 as a new active substance. Under Regulation (EU) 1107/2009 an amended Renewal Assessment Report (RAR) was prepared and published 16 June 2017. Available environmental fate and hazard studies have been considered during this process. The key information pertinent to determining a classification is presented below.

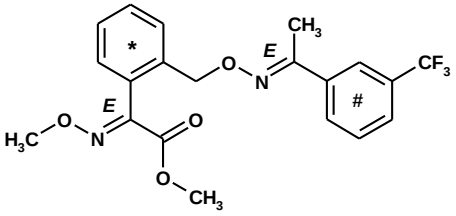
Trifloxystrobin is a fungicide used to protect agricultural and horticultural crops from pathogens. There are two oxime linkages within the molecule, leading to two points of geometric isomerism (Figure 1). Of the potential four isomers of trifloxystrobin: E,E-, E,Z-, Z,E- and Z,Z-. The E,E- isomer is the active substance.

The water solubility of trifloxystrobin at 20 °C is quoted as 0.61 mg/L (Stulz, J. 1997, M-041647-01-1).

A summary of reliable valid information considering the aquatic environmental fate of trifloxystrobin is presented in Table 20 below. Soil data are not presented as suitable aquatic data were available.

Two labelled forms of E,E-trifloxystrobin were assessed in hydrolysis, photolysis and persistence simulation studies presented in this section. Radiolabelled ^{14}C-atoms were located universally in the phenyl groups of phenyl-glyoxylate (GP) and the trifluormethyl-phenyl (TP) functional groups, respectively (Figure 1).

Figure 1: Schematic of trifloxystrobin. Universal labelling of the phenyl groups, phenyl glyoxylate (GP) and trifluormethyl-phenyl (TP) forms is denoted by * and #, respectively.



11.1 Rapid degradability of organic substances

Table 20: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Ready Biodegradation, OECD 301B (1992) Trifloxystrobin (CGA 279202 tech.) GLP	Evolved CO_2 concentrations from the trifloxystrobin (CGA 279202 tech.) samples were identical to that of the untreated inoculum at 28 days Not readily biodegradable	Valid	Weinstock, M., (1994) M-033914-01-1
Hydrolysis of [^{14}C-GP]trifloxystrobin EPA Pesticide Assessment Guidelines, Subdivision N, Section 161-1 GLP	Hydrolytically stable at pH 5 (DT_{50} > 1000 days) DT_{50} – 2.5 days at pH 1 and 25 °C (7.1 days at 12 °C†) DT_{50} - 40 days at pH 7 and 25 °C (60 days at 12 °C†) DT_{50} -1.2 days at pH 9 and 25 °C (3.4 days at 12 °C†) DT_{50} < 0.04 days at pH 13 and 60 °C (1.6 days at 12 °C†)	Valid	Kitschmann, P., (1996) M-033720-01-1

Method	Results	Remarks	Reference
	pH > 5 the hydrolytic degradant CGA 321113 was detected as the major transformation product Hydrolytic degradation of CGA 321113 was assessed at pH 9 and 13 at 60 ºC, yielding DT_{50} values of 240 days and 440 days, respectively (equating to DT_{50} values > 1000 days at 12 ºC for pH 9 and 13[†]). Mineralisation was not measured as part of this study		
Hydrolysis of [^{14}C-TP]trifloxystrobin EPA Pesticide Assessment Guidelines, Subdivision N, Section 161-1 GLP	Hydrolytically stable at pH 5 (DT_{50} > 1000 days) DT_{50} – 3.2 days at pH 1 and 25 ºC (9.1 days at 12 ºC[†]) DT_{50} - 40 days at pH 7 and 25 ºC (113 days at 12 ºC[†]) DT_{50} -2.3 days at pH 9 and 25 ºC (6.5 days at 12 ºC[†]) DT_{50} < 0.04 days at pH 13 and 60 ºC (1.63 days at 12 ºC[†]) pH > 5 the hydrolytic degradant CGA 321113 was detected as the major transformation product Hydrolytic degradation of CGA 321113 was assessed at pH 7, 9 and 13 at 60 ºC, yielding DT_{50} values of 73 days, 150 days and 170 days, respectively (equating to DT_{50} values > 1000 days at 12 ºC for pH 7, 9 and 13[†]). Mineralisation was not measured as part of this study	Valid	Ulbrich, R., (1997a) M-033737-01-1
Aerobic Mineralisation in Surface Water, OECD 309 [^{14}C-GP] Trifloxystrobin, > 98.4% GLP	High concentration (HC) test system (53.7 µg/L) primary degradation DT_{50} at 22.9 °C – 1.41 days (3.3 days when re-calculated to 12 °C[†]) Low concentration (LC) test system (6.1 µg/L) primary degradation DT_{50} at 22.9 °C – 1.36 days (3.4 days when re-calculated to 12 °C) HC: Major transformation product CGA 321113 detected at 42.8% AR (1 day after treatment; DAT) rising to 98.3% AR (62 DAT) LC: Major transformation product CGA 321113 detected at 40.7% AR (1 DAT) rising to 99.4% AR (62 DAT) HC: < 0.1 - 0.3% AR mineralised to CO_2 LC: < 0.1 - 0.2% AR mineralised to CO_2	Valid	Fahrbach, M., (2013) M-449602-01-1
Aerobic Aquatic	Dissipation rates (DT_{50} 19 °C):	Valid	Ulbrich, R., (1997b)

Method	**Results**	**Remarks**	**Reference**
Metabolism BBA guidelines (Richtlinie für Prüfung von Pflanzenschutzmittlen, Teil IV, 5-1, December 1990) [^{14}C-GP]trifloxystrobin GLP	 River sediment/water system: 1.2 days (water) 4.2 days (sediment) 3.5 days (total system) Pond sediment/water system: 1.1 days (water) 1.5 days (sediment) 1.2 days (total system) Dissipation rates (DT_{50} re-calculated to 12 °C): River sediment/water system: 2.1 days (water) 7.4 days (sediment) 6.1 days (total system) Pond sediment/water system: 1.9 days (water) 2.6 days (sediment) 2.1 days (total system) Mineralisation: At study termination $^{14}CO_2$ accounted for maxima of 10.7% AR and 6.2% AR in the river and pond systems, respectively.		M-033922-01-1
Aerobic Aquatic Metabolism BBA guidelines (Richtlinie für Prüfung von Pflanzenschutzmittlen, Teil IV, 5-1, December 1990) [^{14}C-TP]trifloxystrobin GLP	Dissipation rates (DT_{50} 20 °C): River sediment/water system: 1.1 days (water) 3.3 days (sediment) 2.8 days (total system) Pond sediment/water system: 1.1 days (water) 1.9 days (sediment) 1.2 days (total system) Dissipation rates (DT_{50} re-calculated to 12 °C): River sediment/water system: 2.1 days (water) 6.3 days (sediment) 5.3 days (total system) Pond sediment/water system: 2.1 days (water) 3.6 days (sediment) 2.3 days (total system) Mineralisation: At study termination $^{14}CO_2$ accounted for maxima of 9.2% AR and 5.7% AR in the river and pond systems, respectively.	Valid	Kitschmann, P., (1997) M-033933-01-1

Method	**Results**	**Remarks**	**Reference**
Kinetic evaluation of degradation and dissipation behaviour of trifloxystrobin and its metabolite CGA 321113 in water / sediment systems according to FOCUS kinetics using the KinGUI 2 tool, Not GLP	Rapid degradation/dissipation of trifloxystrobin was witnessed in the water, sediment and total system. Calculated modelling DT_{50} for trifloxystrobin (at 20 °C) was 0.76 days, 2.45 days and 1.69 days for water, sediment and total system phases respectively. These values were re-calculated to 12 °C giving 1.44 days, 4.65 days and 3.21 days for water, sediment and total system phases respectively. Degradation of metabolite CGA 321113 was slower, with modelled DT_{50} values (at 20 °C) of 209.7 days, 708.7 days and 388.0 days for water, sediment and total system compartments, respectively. These values were re-calculated to 12 °C giving 398 days, >1000 days and 736 days for water, sediment and total system compartments, respectively.	Re-evaluation of validated data	Reinken, G. and K. Massen (2013) M-468895-01-1

Method	**Results**	**Remarks**	**Reference**
Aqueous Photolysis of [^{14}C-GP-(U)]-trifloxystrobin under laboratory conditions EP Pesticide Assessment Guidelines, Subdivision N, Series 161-2 GLP	Under illuminated experimental conditions at pH 7.2 and 25 °C a primary degradation DT_{50} of 2.7 days was calculated Under dark experimental conditions at pH 7.2 and 25 °C a primary degradation DT_{50} of 36 days was calculated Volatile radioactivity was < 1% AR under all conditions.	Valid	Schäffer, A., (1996) M-033754-02-1
Aqueous Photolysis of [^{14}C-TP-(U)]-trifloxystrobin under laboratory conditions EP Pesticide Assessment Guidelines, Subdivision N, Series 161-2 GLP	Illuminated experimental conditions, pH 7 at 25°C: Run 1 - Radioactive recovery > 47.6% AR, due to volatile loss. Illuminated and dark control primary degradation DT_{50} values for Run 1 are 9.5 days and 26 days, respectively. Run 2 - Radioactive recovery of 88.3 – 112.5% AR. Illuminated and dark control primary degradation DT_{50} values for Run 2 are 5.8 days and 23 days, respectively. Volatile radioactivity at the final sampling interval accounted for < 1% of that applied in each solvent trap for both the illuminated and dark controls samples. Illuminated experimental conditions, pH 5 at 25 °C: Radioactive recovery 97.3 – 104.2% AR. The illuminated primary degradation DT_{50} value was 2.6 days. No discernible degradation was observed in the dark control Volatile radioactivity accounted for 21.9% of that applied, of which 21.5% AR was attributed to CGA 10710 in the toluene traps Volatile radioactivity at the final sampling interval (14 days), associated with butanol, ethylene glycol, sulphuric acid and sodium hydroxide traps accounted for < 1% of radioactivity applied in each trapping solvent, for both the illuminated and dark controls samples.	Valid	Kitschmann, P., (1997) M-033788-01-1

Method	Results	Remarks	Reference
Rate and quantum yield of the direct phototransformation of CGA 279202 under laboratory conditions in water UBA Guidelines ('Phototransformation of chemicals in water, Part A, Direct Phototransformation', Berlin, FRG, Jan 1990 99.7%, ^{12}C-trifloxystrobin GLP	DT_{50} values of trifloxystrobin in shallow natural waters at 40 and 50 °N were calculated to be: 1.3 days and 3.1 days trifloxystrobin alone 17.5 days and 42.2 days trifloxystrobin and isomers	Valid	Phaff, R., (1998) M-033847-02-1
Photolysis of Trifloxystrobin in Natural Water EPA Pesticide Assessment Guidelines, Subdivision N, Section 161-2 (1982), JMAFF New Test Guidelines for Supporting Registration of Chemical Pesticides (2000), SETAC Procedures for assessing the Environmental Fate and Ecotoxicity of Pesticides, Section 10 (1995) Radiochemical purity of ^{14}C-Trifoxystrobin, > 99.9% GLP	DT_{50} of 0.11 days, is equivalent to an estimated environmental half-life of 0.9 days under solar conditions at Tokyo, Japan or 0.4 days under extreme solar conditions at Phoenix, AZ (USA). It is not possible to separate degradation as a result of photolysis from that of hydrolysis within this study. Therefore the DT_{50} of 0.11 days is for hydrolysis AND photolysis and not a DT_{50} for photolysis alone. A mean maximum of 0.4% AR was attributed to $^{14}CO_2$ during irradiation and VOCs were not detected. Neither $^{14}CO_2$, nor VOCs were detected in the dark control samples. All metabolites or isomers indicated a peak and a clear declining trend to the end of study in natural water, even the CGA 321113 degradate.	Valid	Sneikus, J., (2003) M-106330-01-1

[1]Values calculated using modified Arrhenius equation presented in ECHA guidance documents Chapter R.7b: End Specific Guidance, version 4.0 – June 2017 pp 206

11.1.1 Ready biodegradability

Study 1 – Weinstock, M., (1994), M-033914-01-1

In compliance with GLP standards, ready biodegradation of trifloxystrobin was studied according to OECD guideline 301 B (1992).

Activated sewage sludge was added to a standard mineral solution and preincubated overnight at 22 °C. Duplicate samples of inocula (1.2 litres) were treated with unlabelled trifloxystrobin (26-27.2 mg/L equivalent

to 15.3-16 mg ThOC/L), sodium benzoate (15 mg DOC/L) or both. Further samples of untreated inocula were prepared. All samples were incubated at 22°C for 29 days in flasks fitted with $^{14}CO_2$ traps (NaOH).

For all treatments, evolved CO_2 was determined by carbon analysis at day 0 and on ten further sampling intervals. At study termination, approximately complete conversion of sodium benzoate to CO_2 was observed, with and without trifloxystrobin. The ^{14}C sodium benzoate control dosed with ^{12}C trifloxystrobin was the toxicity control. Evolved CO_2 from the trifloxystrobin samples was indistinguishable from untreated inocula. Hence, trifloxystrobin is classified as 'not readily biodegradable'.

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

Study 1 – Kitschmann, P., (1996), M-033720-01-1

In compliance with GLP standards, hydrolytic stabilities of [^{14}C-GP] - trifloxystrobin were assessed according to EPA guidelines (Pesticide Assessment Guidelines, Subdivision N, Section 161-1).

Analytical recoveries were in the range of 86.7-105.5% Applied Radioactivity (AR) for [^{14}C-GP] -trifloxystrobin hydrolysis samples. Measured concentrations of [^{14}C-GP] - trifloxystrobin were used to calculate first order DT_{50} values. These are presented in Table 20.

Table 21: **Summary of Experimental and Calculated first-order Hydrolytic Half-lives (DT_{50}) for [^{14}C-GP] trifloxystrobin**

DT_{50} [^{14}C-GP] trifloxystrobin (days)				
pH	**60 ºC Experimental**	**25 ºC Experimental**	**20 ºC Calculated**	**12 ºC Calculated**
1	-	2.5	3.8	7.1
5	-	480	716	> 1000
7	-	40	59.7	113.2
9	-	1.2	1.8	3.4
13	< 0.04	-	< 1.0	< 1.9

Rates of hydrolysis at 12 and 20 °C have been calculated using Arrhenius parameters based on the experimentally obtained rate constants at 25 and 60 °C (ECHA, 2017)

The major degradation product generated at pH 5 and above was CGA 321113. DT_{50} values for CGA 321113 were determined for pH 7, 9 and 13 at 60°C, for [^{14}C-GP] - trifloxystrobin samples. $DT_{50\ (CGA\ 321113)}$ calculated for 12, 20 and 25 °C were > 1000 days. Hydrolytic degradation of trifloxystrobin is pH dependent. For each temperature assessed, rates of degradation decrease as the pH increases from 1 and 5, where minima rates were observed. Rates of degradation increase as the pH increases from pH 5 to pH 13, where the fastest hydrolytic degradation rates were observed.

Measurement of evolved $^{14}CO_2$ and volatile organic carbon moieties were not included in the study design.

Study 2 – Ulbricht, R., (1997a), M-033737-01-1

In compliance with GLP standards, hydrolytic stabilities of [^{14}C-TP] - trifloxystrobin were assessed according to EPA guidelines (Pesticide Assessment Guidelines, Subdivision N, Section 161-1).

Analytical recoveries were in the range of 88.9-144.9% Applied Radioactivity (AR) for [^{14}C-TP] - trifloxystrobin hydrolysis samples. Measured concentrations of [^{14}C-TP] - trifloxystrobin were used to calculate first order DT_{50} values. These are presented in Table 22.

Table 22: Summary of Experimental and Calculated first-order Hydrolytic Half-lives (DT_{50}) for [^{14}C-TP] trifloxystrobin

DT_{50} [^{14}C-TP] trifloxystrobin (days)				
pH	**60 °C Experimental**	**25 °C Experimental**	**20 °C Calculated**	**12 °C Calculated**
1	-	3.2	4.8	9.1
5	-	> 1000	> 1000	> 1000
7	-	40	59.7	113.2
9	-	2.3	3.4	6.5
13	< 0.04	-	< 1.0	< 1.9

Rates of hydrolysis at 12 and 20 °C have been calculated using Arrhenius parameters based on the experimentally obtained rate constants at 25 and 60 °C (ECHA, 2017)

The major degradation product generated at pH 5 and above was CGA 321113. DT_{50} values for CGA 321113 were determined for pH 9 and 13 at 60 °C, [^{14}C-TP] - trifloxystrobin samples, $DT_{50\ (CGA\ 321113)}$ calculated for 12, 20 and 25 °C were > 1000 days.

Measurement of evolved $^{14}CO_2$ and volatile organic carbon moieties were not included in the study design. Mean radioactive recoveries from the closed test systems lay within the desired range of 90 - 110% AR. Losses and variations were proposed to be caused by sorption of the test item to the glass walls of the test vessels.

11.1.4 Other convincing scientific evidence

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

No relevant data.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No relevant data.

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

Please note that soil data have not been presented as suitable aquatic data are available.

Study 1 – Fahrbach, M., (2013), M-449602-01-1

Trifloxystrobin was assessed in an aerobic mineralisation in surface water simulation study, which followed OECD Test Guideline 309, and was compliant with GLP standards. Transformation and mineralisation of [^{14}C-GP] trifloxystrobin at a measured mean radiochemical purity of > 98.4% were studied under pelagic conditions in the absence of sediment. Exposure was performed in the dark for 62 days and an average temperature of 22.9 °C. Table 23 presents the physico-chemical properties and characteristics of the surface water.

Table 23: Physico-chemical Properties and Characteristics of the Surface Water

Parameter	Results/Units
Water Designation	Froeschweiher Pond

Origin	Moehlin AG, Switzerland
GPS Coordinates	47º32' N; 007º48' E
Date of Sampling	27/08/2012
Sampling Depth	30 cm
Storage Time	24 hours between sampling and study commencement
Storage Temperature (ºC)	4
pH †	8.2
Redox Potential E_H (mV) †	466
Oxygen Content (mg/L) †	10.6
Hardness (ºdH) †	14.6
BOD (mg/L)	<5.0
DOC (mg C/L)	21.3
TOC (mg C/L)	22.8
Total Nitrogen (mg/L)	2.22
Total Phosphorous (mg/L)	0.45
Total Nitrate (mg/L)	1.31
Total Nitrite (mg/L)	1.23
Total Ammonium (mg/L)	1.98
Dissolved Orthophosphate (mg/L)	0.01

† Parameters measured at sampling site

Concentrations of 6.1 µg/L and 53.7 µg/L (0.061 mg/L and 0.537 mg/L) trifloxystrobin were used for low and high concentration test systems respectively- both values are within maximum concentrations set out by OECD 309. The incubation period of trifloxystrobin was 62 days, during which eight sampling intervals were performed (0, 1, 2, 4, 7, 14, 28 and 62 days after treatment; DAT).

A sterilised control (53.7 µg/L trifloxystrobin; 0.537 mg/L) was established at the start of the study to examine abiotic degradation of the test item. This was sampled only at the end of the full 62 days incubation period. Sterilisation was performed by autoclaving at 121 ºC for 20 min.

Microbial viability of the test system was assessed using a reference substance concurrently with the trifloxystrobin study, for a period of 14 days (sampled on days 0, 3 and 14). In total, ten reference control systems were established each using [ring-^{14}C(U)] Benzoic acid (radiochemical purity > 95.4%) at a concentration of 11.1 µg/L (0.111 mg/L); the mineralisation DT_{50} of the reference substance was < 3 days.

Radioactive recoveries for the low concentration test system ranged from 96.0 to 102.2% AR (mean material balance 98.2% AR). Radioactive recoveries for the high concentration test system ranged from 95.5 to 98.3% AR (mean material balance 96.7% AR).

Under aerobic conditions, trifloxystrobin rapidly hydrolyses to form metabolite CGA 321113. Very little mineralisation occurred during the study and the concentration of unidentified residues was not significant. Sterilised test vessels showed similar degradation of trifloxystrobin/formation of CGA 321113 as non-sterilised test systems, implying that the degradation of trifloxystrobin in aerobic surface waters is an abiotic process.

Maximum $^{14}CO_2$ formation was seen on day 28 for both high and low concentration test systems, 0.2 % and 0.3% AR, respectively. The formation of volatile organic compounds was < 0.1% AR in both the high and low test systems.

Review of the study under Directive 91/414/EEC included re-fitting of the high and low concentration results using CAKE software ordinary least squared (OLS) parameter optimization) to generate DT_{50} values. The results were practically identical to those of study calculations (derived via KinGUI). For both the high and low concentration test systems, single-first order (SFO) was selected as being the most appropriate kinetic fate model for trifloxystrobin. SFO fits, for both high and low concentrations showed a potential (but insignificant) systematic error in that concentrations are consistently under predicted. However, this frequently occurs after > 90% primary degradation of the substance has been exceeded. Subsequently, SFO primary degradation DT_{50} values (19 °C) of 1.36 days (high concentration) and 1.41 days (low concentration) were calculated. For the purpose of classification these primary degradation values have been converted to 12 °C, in line with ECHA guidance and Member State Committee testing protocols, to reflect a more environmentally relevant temperature:.

- High Concentration primary degradation DT_{50} - 3.4 days at 12 °C
- Low Concentration primary degradation DT_{50} 3.3 days at 12 °C

Study 2 - Ulbrich, R., (1997b), M-033922-01-1

In compliance with GLP standards, an aerobic sediment/water study examining [^{14}C-GP] trifloxystrobin was conducted according to BBA guidelines (Richtlinie für Prüfung von Pflanzenschutzmittlen, Teil IV, 5-1, December 1990. Swiss Rhine river and pond water (500 mL, 6 cm collection depth) and associated sandy silt loam and clay loam sediments (139 -145 g dry weight, 2 - 2.5 cm collection depth) were used to prepare the test systems. Table 24 presents the physico-chemical properties and characteristics of the test sediments.

Table 24: Physico-chemical Properties and Characteristics of the Test Sediments

Test System	% Clay	% Silt	% Sand	% Organic Carbon	pH	Microbial Biomass mg orgC/100g
River	15.2	45.6	39.2	2.1	7.5	207
Pond	26.0	41.4	32.6	2.6	7.3	177

[^{14}C-GP] trifloxystrobin was applied at 0.3 mg /L. All flasks were incubated at 19°C in the dark for up to 205 days.

Dissolved oxygen remained above 8 mg/L in both systems throughout the study and pH remained constant at 7.7 and 8.2 for the river and pond systems respectively. Mean redox potential was -410±12 to -412±16 mV and -389±8.0 to -401±5.0 mV, and 180±15 to 223±22 mV and 209±3.0 to 212±32 mV in the sediment and water of the river and pond systems respectively.

Radioactive recoveries were 87 - 102.5% AR (mean 99.3% AR) and 98 - 105% AR (mean 101.8% AR) in the river and pond systems, respectively. Trifloxystrobin was detected in sediments at maximum concentrations of 36.6 and 13.4% AR after 1 DAT in the river and pond systems, respectively. A single major metabolite was detected, CGA 321113, accounting for a maximum of 52.9 and 76.9% AR in the water phase (7 days after treatment), 51.1 and 42.7% AR in the sediment (21 days after treatment) and 93.5 and 100.7% AR in total, in the river and pond systems, respectively.

Unextractable radioactivity reached maxima of 12.3% AR in river and 13.8% AR in pond systems at study termination. The only volatile detected was $^{14}CO_2$ at 10.7% AR in the river system and 6.2% AR in the pond system at study termination.

The study reported the rates of dissipation (DT_{50}) of [^{14}C-GP] trifloxystrobin, which transformed to the primary degradant CGA 321113 at 19 °C. For the purpose of classification these values have been converted to 12 °C, in line with ECHA guidance and Member State Committee testing protocols, to reflect a more environmentally

relevant temperature. Proposed degradation pathway of trifloxystrobin in water and sediment is presented in Figure 2 (amended RAR, 2017).

Data are presented in Table 25.

Table 25: Rates of Dissipation of [^{14}C-GP] trifloxystrobin and the primary transformation product CGA 321113 in River and Pond Test Systems at 20 °C and converted to 12 °C

Test System and Compartment	[^{14}C-GP]trifloxystrobin		CGA 321113	
	DT_{50} (days) at 20 °C	DT_{50} (days) at 12 °C	DT_{50} (days) at 20 °C	DT_{50} (days) at 12 °C
River water	1.2	2.1	320	560
River sediment	4.2	7.4	>1000	>1000
River system total	3.5	6.1	>1000	>1000
Pond water	1.1	1.9	170	298
Pond sediment	1.5	2.6	not detected	-
Pond system total	1.2	2.1	360	>1000

Study 3 - Kitschmann, P. (1997) M-033788-01-1

In compliance with GLP standards, an aerobic sediment/water study examining [^{14}C-TP] trifloxystrobin was conducted according to BBA guidelines (Richtlinie für Prüfung von Pflanzenschutzmittlen, Teil IV, 5-1, December 1990.

[^{14}C-GP] trifloxystrobin was applied to separate flasks at an application rate of 0.3 mg mg/L. All flasks were incubated at 20 °C in the dark for up to 214 d.

Dissolved oxygen remained above 6.4 mg/L throughout the study and pH remained constant around 8. Mean redox potential was -402±26 to -414±14 mV and -393±13 to -382±30 mV, and 189±19 to 198±19 mV and 215±24 to 241±18 mV in the sediment and water of the river and pond systems respectively.

Radioactive recoveries were 96 – 101.7% AR (mean 99.2% AR) and 96 – 101.9% AR (mean 98.8% AR) in the river and pond systems, respectively. Trifloxystrobin was detected in sediments at maximum concentrations of 42.3 and 10% AR 1 DAT in the river and pond systems, respectively. A single major metabolite was detected, CGA 321113, accounting for a maximum of 41.3 and 72.2% AR in the water phase (28 and 4 DAT), 48.4 and 47.1% AR in the sediment (28 and 100 DAT) and 89.7 and 93.8% AR in total, in the river and pond systems, respectively. Proposed degradation pathway of trifloxystrobin in water and sediment is presented in Figure 2 (amended RAR, 2017).

Unextractable radioactivity reached maxima of 12.9% AR in river and 14.9% AR in pond systems at study termination. $^{14}CO_2$ was the only volatile detected and accounted for 9.2% AR in the river system and 5.7 % AR in the pond system at study termination.

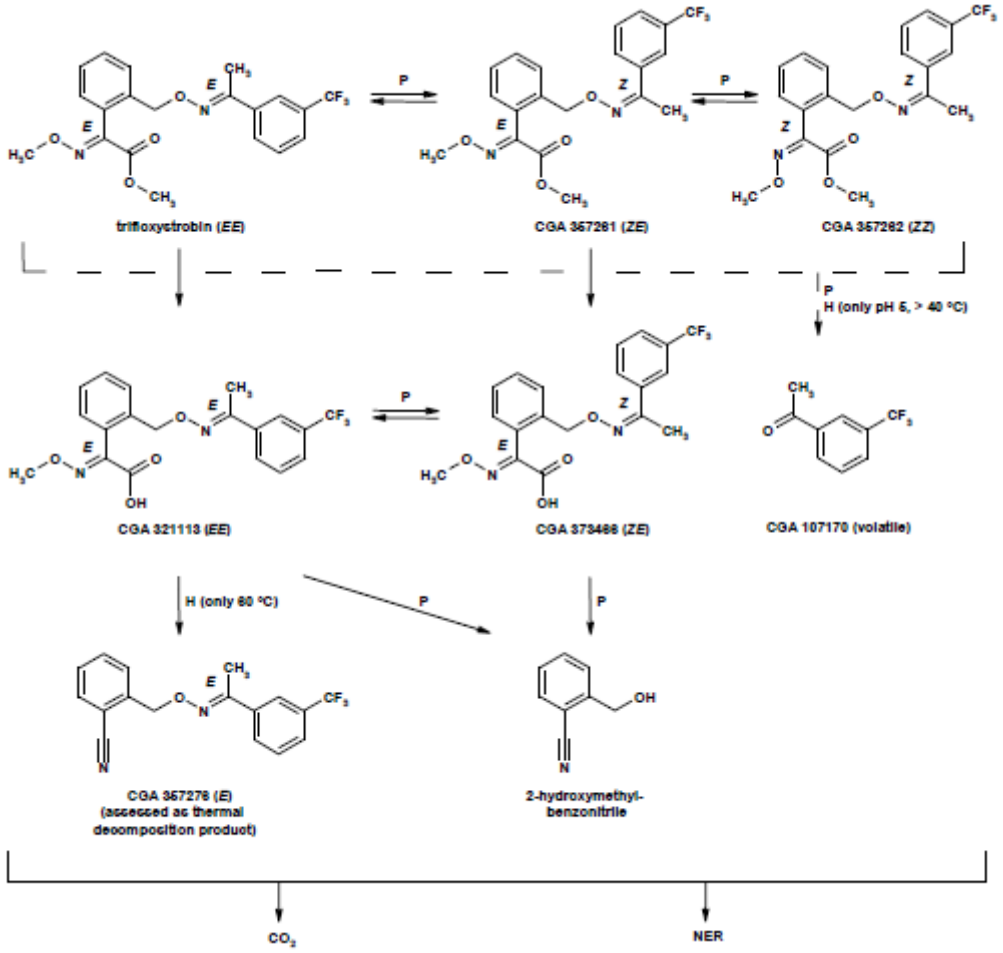
The study reported the rates of dissipation (DT_{50}) of [^{14}C-TP] trifloxystrobin and the primary transformation product CGA 321113 at 20 °C. For the purpose of classification these values have been converted to 12 °C, in line with ECHA guidance and Member State Committee testing protocols, to reflect a more environmentally relevant temperature. Data are presented in Table 26.

Table 26: Rates of Dissipation of [^{14}C-TP] trifloxystrobin and the primary transformation product CGA 321113 in River and Pond Test Systems at 20 °C and converted to 12 °C

Test System and Compartment	[^{14}C-TP]trifloxystrobin		CGA 321113	
	DT_{50} (days) at 20 °C	DT_{50} (days) at 12 °C	DT_{50} (days) at 20 °C	DT_{50} (days) at 12 °C
River water	1.1	2.1	310	588

Test System and Compartment	[^{14}C-TP]trifloxystrobin		CGA 321113	
	DT_{50} (days) at 20 °C	**DT_{50} (days) at 12 °C**	**DT_{50} (days) at 20 °C**	**DT_{50} (days) at 12 °C**
River sediment	3.3	6.3	460	872
River system total	2.8	5.3	380	721
Pond water	1.1	2.1	180	341
Pond sediment	1.9	3.6	n.d.	-
Pond system total	1.2	2.3	480	910

Figure 2: Proposed degradation pathway of trifloxystrobin in water and sediment (amended RAR, 2017)



Study 4 – Reinken, G. and K. Maassen (2013), M-468895-01-1

Kinetic re-evaluation of data generated from two aerobic water-sediment studies, Ulbrich, R., (1997b) and Kitschmann, P. (1997g) were performed due to updates in the FOCUS guidance (published 2006; updated 2011) using KinGUI 2 and CAKE software by Reinken, G. and K. Maassen, (2013).

Degradation and dissipation of trifloxystrobin and its main metabolite (CGA 321113) in the aquatic environment were investigated by the Applicant by kinetic evaluation of the data using FOCUS guidance (2006) and KinGUI 2 software. Assessment for Directive 91/414/EEC involved re-fitting all data using CAKE software utilising OLS optimisation. This generated almost identical results to those of the original assessment. Negligible differences were observed between the KinGUI (IRLS) and CAKE (with OLS) fitting. In all test systems, the DT_{50} for trifloxystrobin was within the study duration and declines well described. With few

exceptions, the DT_{50} of CGA 321113 exceeded study incubation period, and as such the reliability of the calculated DT_{50} values should be treated cautiously.

Data from the two studies were examined using different models (SFO, FOMC, DFOP and HS) to generate end points for trifloxystrobin and CGA 321113 degradation/dissipation in all phases (water, sediment and total system), and also trigger evaluation end points for total system dissipation for both trifloxystrobin and CGA 321113.

SFO fits were visually and statistically acceptable for modelling purposes. Individually, the water and sediment phases indicated bi-phasic primary degradation was occurring. Therefore FOMC, DFOP and HS kinetic models were investigated as trigger evaluation end points as these offered a further improved visual and statistical fits. Selection of bi-phasic models over the simple SFO model for trigger evaluation end points was a result of following the steps laid out in the FOCUS (2006) guidance document.

A summary table (Table 27) of modelling and triggering end points for trifloxystrobin and CGA 321113 is presented below. For the purpose of classification these values have been converted to 12 °C, in line with ECHA guidance and Member State Committee testing protocols, to reflect a more environmentally relevant temperature.

Table 27: Summary of Modelling and Trigger End Points for Trifloxystrobin and CGA 321113 at 12 °C

Compartment	Modelling	Trigger
DT_{50} Trifloxystrobin (days)		
Total System	3.2	3.2
Water	1.4	1.4
Sediment	4.6	4.4
DT_{50} CGA 321113 (days)		
Total System	736	735
Water	398	329
Sediment	> 1000	> 1000

11.1.4.4 Photochemical degradation

Study 1 – Schäffer, A., (1996), M-033754-02-1

In compliance with GLP, the aqueous photolysis of [^{14}C-GP] trifloxystrobin was studied according to EPA guidelines (Pesticide Assessment Guidelines, Subdivision N, Series 161-2).

Radioactive recoveries were 97 - 101.6% AR (photolysis in aqueous solution). After 30 days, [^{14}C-GP] trifloxystrobin accounted for a mean 9.3% AR and 54.6% AR in illuminated and dark control samples, respectively. First order primary degradation DT_{50} values were calculated as 2.7 days under illuminated conditions, and 36 days under dark conditions at 25 °C.

Decline under illuminated conditions was stated by the study authors to be equivalent to a DT_{50} of 1.3 days at a latitude of 40°N in mid-summer conditions (a two compartment model was proposed with respective DT_{50} of 0.8 and 13.5 days.

Under illuminated conditions CGA 357262 and CGA 357261 were detected at maximums of 10.2 and 40% AR respectively. These are isomers of trifloxystrobin. Fractions M10, M20 and M50 were also detected at maximums of 20.4, 10.4 and 16.9% AR respectively. M10 and M20 were identified as heterogeneous mixtures of various polar products, each ≤ 5% AR. M50 was identified as an isomer of CGA 321113 (CGA 373466 is the proposed structure for M50). All other fractions were < 10% AR.

Under dark conditions the sole major metabolite was CGA 321113 at a maximum of 40.77 % AR, other fractions were < 2% AR. Volatile radioactivity was < 1% AR under all conditions.

Study 2 - Kitschmann, P., (1997), M-033788-01-1

In compliance with GLP, the aqueous photolysis of [^{14}C-TP] trifloxystrobin was studied according to EPA guidelines (Pesticide Assessment Guidelines, Subdivision N, Series 161-2).

Total recoveries were 88.5-113.2% AR (dark controls), 97.3-104.2% AR (pH 5). Recoveries in the pH 7 samples were low in Run 1, 47.6% AR, which was proposed to be due to the loss of volatiles. Run 2 at pH 7 was performed with cooled toluene and butanol volatile traps incorporated into the test systems. Recoveries using this system increased to lie within 88.3-112.5% AR.

[^{14}C-TP] trifloxystrobin in the illuminated pH 7 samples, accounted for a 10.2% AR at 691 hrs and 1.9% AR at 763 hrs, for Run 1 and Run 2, respectively. [^{14}C-TP] trifloxystrobin in the dark control pH 7 samples, accounted for a 41.9% AR at 691 hrs and 38.2% AR at 763 hrs, for Run 1 and Run 2, respectively.

First order-DT_{50} at pH 7 (25 °C) were calculated for and are presented in Table 28.

Table 28: DT_{50} values at 25 °C for the Aqueous Photolysis of [^{14}C-TP] trifloxystrobin

Run	DT_{50} [^{14}C-TP] trifloxystrobin (days)	
	Illuminated	Dark
pH 7 Run 1	9.5	26
pH 7 Run 2	5.8	23
pH 5	2.6	No discernible degradation

Decline under illuminated conditions was stated to be the equivalent to a DT_{50} of 2.6 days at a latitude of 40 °N in mid-summer conditions.

Illuminated pH 5 samples contained CGA 357261, which was detected at a maximum of 41.6% AR, all other metabolites were at < 10% AR. At the final sampling interval, a maximum mean 54.4% AR was located in the volatile traps. Of the 54.4% a mean 53.8% AR was found in the toluene traps and was identified as CGA 107170. Mean radioactive content located in the ethylene glycol and the sodium hydroxide traps accounted for < 0.2 % and < 0.5 %, respectively of that applied.

Illuminated pH 7 samples from Run 1 (radioactive recoveries were as low as 47.6 %) contained CGA 357261 and CGA 373466 were detected at maxima of 40.5 and 13% AR respectively, and other metabolites accounted for < 8.5% AR. Volatiles accounted for 5.1% AR.

Illuminated pH 7 samples generated during Run 2 contained CGA 373466, CGA 321113 and CGA 357261, which were detected at maximums of 44.1, 23.0 and 35.0% AR respectively, other metabolites accounted for a total of < 7% AR. Radioactivity recovered in the volatile traps accounted for 21.9 % AR, the majority at 21.4% AR was identified as CGA 107170 in the toluene trap.

Volatile radioactivity at the final sampling interval (14 days), associated with butanol, ethylene glycol, sulphuric acid and sodium hydroxide traps accounted for < 1 % of that applied in each solvent for both the illuminated and dark controls samples.

Study 3 – Phaff, R. (1998), M-033847-02-1

In compliance with GLP photolytic degradation rate and quantum yield of trifloxystrobin were calculated according to UBA guidelines ('Phototransformation of chemicals in water, Part A, Direct Phototransformation', Berlin, FRG, January 1990).

A decline curve was fitted to the experimental data and a mean DT_{50} of 119 minutes calculated for total trifloxystrobin (including isomers), a mean DT_{50} of 10 minute was calculated for trifloxystrobin alone.

The UV/VIS absorption spectra of 100 mL of pH 7 buffer with 30% acetonitrile containing 10.6 ppm trifloxystrobin and 10 mL acetonitrile containing 1060 ppm trifloxystrobin were determined. The decadic molar extinction coefficients were calculated and spectral data averaged for wavelength intervals of 2 mm.

The UV/VIS absorption spectra of trifloxystrobin in pH 7 buffer with acetonitrile was characterised by an absorption band with a decadic molar extinction coefficient of 16,297 [L mol^{-1} cm^{-1}] at its maximum of 249.5 nm, which declined to 320 nm. There was therefore a spectral overlap with sunlight of 297.5 – 320.0 nm, which indicated a potential for photolytic degradation. For the purpose of calculations spectra of trifloxystrobin isomers were considered identical to trifloxystrobin alone.

Quantum yields (Φ; dimensionless) of the sum of trifloxystrobin and its isomers was calculated as 0.0639 and that of trifloxystrobin alone was 0.2272. Thus the majority of absorbed light energy is utilised for cis-trans isomerisation.

DT_{50} values of trifloxystrobin in shallow natural waters at 40 and 50 °N were subsequently calculated to be 1.3 days and 3.1 days, respectively, for trifloxystrobin alone and 17.5 days and 42.2 days, respectively, for trifloxystrobin and isomers.

Study 4 – Sneikus, J. (2003), M-106330-01-1

In compliance with GLP the indirect photolysis [benzene acetic-UL-^{14}C] trifloxystrobin ([^{14}C-GP]trifloxystrobin) in natural water was examined according to EPA Pesticide Assessment Guidelines, Subdivision N, Section 161-2 (1982), JMAFF New Test Guidelines for Supporting Registration of Chemical Pesticides (2000) and SETAC Procedures for assessing the Environmental Fate and Ecotoxicity of Pesticides, Section 10 (1995).

The irradiance from the xenon light was compared to natural sunlight. The total test period of continuous light exposure, 8 days, was equivalent to 61.9 solar days at Tokyo/Japan and 29.9 solar summer days at Phoenix, Arizona/USA.

Phototransformation of trifloxystrobin (E,E-isomer) started with isomerisation to E,Z- (CGA 331409), Z,E-(CGA 357261) and Z,Z-(CGA375262) isomers. These transformation products degraded to CGA 321113 (trifloxystrobin acid, E, E-isomer) and related isomers (CGA 373466, CGA 373465). Further phototransformation generated a multitude of minor polar products.

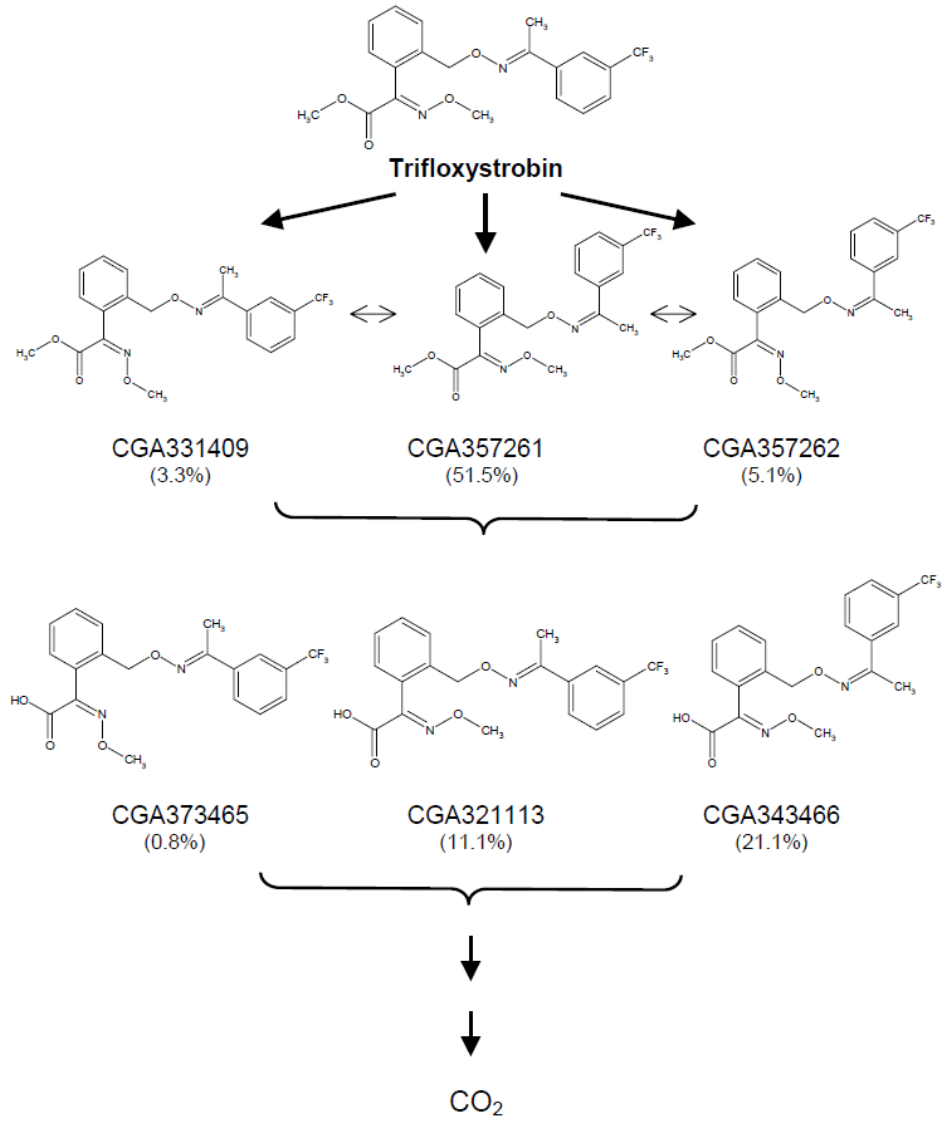
The mean average recovery of radioactivity from irradiated samples was 101.2% AR with a relative standard deviation of 4.1% AR. The mean recovery of the dark samples was 106.2 % AR.

A maximum of 0.4% AR was attributed to $^{14}CO_2$ during irradiation and VOCs were not detected. Neither $^{14}CO_2$, nor VOCs were detected in the dark control samples.

All metabolites and/or isomers indicated a peak and clear declining trend to the end of study in natural water, including the CGA 321113 degradate.

The proposed pathway of indirect photolysis of trifloxystrobin in natural water is presented in Figure 3

Figure 3: Proposed pathway of indirect photolysis of trifloxystrobin in natural water (Sneikus, J.; 2003)



values in brackets: max. % of applied radioactivity

Trifloxystrobin degraded with an experimental half-life of 0.11 days (hockey-stick fit)

Based on the irradiance of the xenon light used in the tests, the experimental DT_{50} of 0.11 days is equivalent to an estimated environmental half-life of 0.9 days under solar conditions Tokyo, Japan or 0.4 days under extreme solar conditions Phoenix, AZ (USA).

Due to the absence of data for dark samples at any time point except day 8 it is not possible to determine the DT_{50} for the dark samples. This means it is not possible to separate degradation as a result of photolysis from that of hydrolysis within this study. Therefore the DT_{50} of 0.11 days is for hydrolysis and photolysis and not a DT_{50} for photolysis alone.

11.2 Environmental fate and other relevant information

Adsorption/Desorption

Study 1 – Schäffer, A., (1995), M-033549-03-1

In compliance with GLP a batch equilibrium adsorption/desorption study was conducted for trifloxystrobin according to EPA guidelines (Pesticide Assessment Guidelines Subdivision N, Series 163-1, 1982).

The adsorption and desorption isotherms for each concentration were used to calculate Freundlich coefficients (K_f) and K_{oc} values for each soil, which are given in Table 29 for adsorption. Both desorption steps gave similar K_{oc} values, in the range 2115 - 3522.

Table 29: Adsorption coefficients for trifloxystrobin to five soils.

Soil type	% oc	pH	1/n	K_f	K_{oc}
Collombey loamy sand	0.8	7.3	0.92	14.7	1837
Speyer 2.1 sand	0.3	6.8	1.00	11.2	3745
Gartenacker loam	2.0	7.1	0.94	40.6	2031
Vetroz silt loam	4.7	7.2	0.98	126.1	2683
Illarsaz humic silt loam	19.8	6.7	0.97	325.0	1642

Study 2 - Glänzel, A., (2000), M-049477-01-1

In compliance with GLP standards a batch equilibrium adsorption/desorption study was conducted for trifloxystrobin according to OECD (test guideline 106, 2000) and EPA guidelines (Pesticide Assessment Guidelines Subdivision N, Series 163-1, 1982).

The adsorption and desorption isotherms for HPLC measured trifloxystrobin concentrations for each concentration, were used to calculate Freundlich coefficients (K_f) and K_{oc} values, which are given in Table 30 for adsorption. The desorption step gave a K_{oc} value of 2625 mL/g (equilibrium was not reached due to degradation).

Table 30: Adsorption coefficient for trifloxystrobin

Soil type	% oc	pH	1/n	K_f (mL/g)	K_{oc}(mL/g)
Borstel loamy sand	1.0	5.1	0.94	23.3	2327

Summary

Overall, trifloxystrobin is considered to be slightly mobile in soil (according to FAO, 2000; USEPA, 2006)

Additional Studies:

Additional adsorption studies have been performed on transformation products of trifloxystrobin. The studies are summarised in the following table (Table 31).

Table 31: Summary of adsorption studies performed on the transformation products of trifloxystrobin

Study Author	Guideline	GLP	Transformation Product	Mobility[†]
Schäffer, 1995, M-033569-02-1	1	Yes	CGA 321113	Mobile/Moderately mobile
Glänzel, 2000 M-051381-01-1	1,2	Yes	CGA 321113	Moderately mobile
Heim & Velagaleti, 1997 M-036332-01-1	1	Yes	CGA 373466	Mobile/Moderately mobile
Adam, 2000 M-046346-01-1	1,2	Yes	NOA 413161	Highly mobile
Heim & Velagaleti, 1997 M-036399-01-1	1	Yes	CGA 357261	Moderately mobile
Stroech & Weuthen, 2013 M-447879-01-1	1,3,4,5	Yes	BCS-CU98569	Mobile/Moderately mobile
Tinnefield, 2010 M-361829-01-1	2,4	Yes	NOA 413161	Highly mobile/Moderately mobile
Tinnefield, 2010 M-361835-01-1	2,4	Yes	NOA 413163	Highly mobile/Moderately mobile
Heim & Velagaleti, 1997 M-036507-01-1	1	Yes	CGA 357276	Slightly mobile/Hardly mobile
Stroech & Weuthen, 2012 M-442865-01-1	1,3,4,5	Yes	NOA 409480	Moderately mobile

[†]Classified as per FAO (2002) and US EPA (2006)

1 EPA Pesticide Assessment Guidelines Subdivision N, Series 163-1, 1982

2 OECD Test Guideline 106 (2000) Adsorption –Desorption Using a Batch Equilibrium Method

3 Draft SANCO 11802/2010/rev 1 in accordance with Regulation (EC) No – 1107/2009

4 US EPA OCSPP Test Guideline No. 835.1230

5 Canadian PMRA Guideline DACO 8.2.4.2

Henry's Law Constant:

A calculated Henry's Law Constant was calculated to be 2.3 x 10^{-3} Pa at 25 ^{o}C (Burkhard, N., 1997 M-041515-01-1). Overall, trifloxystrobin is unlikely to partition to air.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON TRIFLOXYSTROBIN (ISO); METHYL (*E*)-METHOXYIMINO-{(*E*)-A-[1-(A,A,A-TRIFLUORO-*M*-TOLYL) ETHYLIDENEAMINOOXY] -*O*-TOLYL}ACETATE

Summary of Fate Information

Two labelled forms of trifloxystrobin were assessed in hydrolysis, photolysis and persistence simulation studies, with the exception of the mineralisation study (OECD TG 309), which examined only one radiolabel. Non-radiolabelled trifloxystrobin was assessed in a ready biodegradation study and a quantum yield determination study.

Ready biodegradation:

Evolved CO_2 from non-radiolabelled trifloxystrobin dosed vessels was indistinguishable from that of control vessels. Trifloxystrobin is therefore classified as 'not readily biodegradable'.

Hydrolysis:

Hydrolytic degradation of trifloxystrobin (EE) in sterile aqueous buffer solutions in the dark in the laboratory is strongly dependent on the temperature and the pH value. Measured concentrations of [^{14}C-GP] - and [^{14}C-TP] - trifloxystrobin were used to calculate first order DT_{50} values. For each temperature assessed, rates of degradation decrease as the pH increases from 1 and 5, where minima rates were observed. Rates of degradation increase as the pH increases from pH 5 to pH 13, where the fastest hydrolytic degradation rates were observed. The major primary degradation product generated at pH 5 and above was CGA 321113. Data from the hydrolysis studies indicated that the longest half-life of trifloxystrobin within the pH range 4 to 9 was in excess of 16 days when adjusted via calculation to 12 °C. The longest half-life for CGA 321113 within the pH range 4 to 9 was in excess of 1000 days when adjusted via calculation to 12 °C.

Trifloxystrobin rapidly hydrolyses (DT_{50} ~ 3.3 days at 12 °C) to form metabolite CGA 321113. Very little mineralisation occurred during the study and the concentration of unidentified residues was not significant. Sterilised test vessels showed similar degradation of trifloxystrobin/formation of CGA 321113 as non-sterilised test systems, implying that the degradation of trifloxystrobin in aerobic surface waters is an abiotic process.

Aerobic Sediment and Water:

Trifloxystrobin (EE) undergoes rapidly primary degradation in water and sediment to the major degradation product CGA 321113 (EE), non-extractable residues and low levels of CO_2.

For [^{14}C-GP] trifloxystrobin unextractable radioactivity reached maxima of 12.3 % AR in river and 13.8 % AR in pond systems at study termination. The only volatile detected was $^{14}CO_2$ at 10.7% AR in the river system and 6.2% AR in the pond system at study termination. For [^{14}C-TP] trifloxystrobin unextractable radioactivity accounted for 12.9% AR in river and 14.9% AR in pond systems at study termination. $^{14}CO_2$ was the only volatile detected and accounted for 9.2% AR in the river system and 5.7 % AR in the pond system at study termination. Data from the two aerobic sediment-water studies combined and statistically examined yielded the following DT_{50} values for trifloxystrobin and CGA 321113 at 12 °C. For trifloxystrobin DT_{50} values were calculated as 1.44 days, 4.65 days and 3.21 days for water, sediment and total system phases respectively. For CGA 321113, DT_{50} values were calculated as 398 days, >1000 days and 736 days for water, sediment and total system phases respectively.

Photolysis studies:

Under photolytic conditions in the laboratory in sterile buffers at pH 5 and pH 7 and in sterile natural water, trifloxystrobin (EE) was rapidly degraded (DT50 ≤ 1.7 days) by E/Z isomerization (in this summary referred to as "photodegradation products"). Trifloxystrobin (EE) isomerized to its major E/Z isomers CGA 357261 (ZE) with max. 51.5% AR (natural water) and CGA 357262 (ZZ) with max. 10.1% AR (buffer pH 7). Trifloxystrobin (EE) and its E/Z isomers were degraded to the major degradation product CGA 321113 (EE) with max. 57.4% AR (natural water) and its major E/Z isomer CGA 373466 (ZE) with max. 21.1% AR (natural water) by hydrolytic ester cleavage and E/Z isomerization. Furthermore, the major volatile degradation product

CGA 107170 was formed with a maximum amount of 53.8% AR (buffer pH 5) by cleavage of the bridge between the aromatic ring systems. Formation of carbon dioxide was very low with a maximum amount of 0.5% AR. A similar process was observed for CGA 321113 (EE) in sterile buffer at pH 5. CGA 321113 (EE) was rapidly degraded (DT50 ≤ 1.7 days) by E/Z isomerization to its major E/Z isomer CGA 373466 (ZE) with max. 60.5% AR. Furthermore, the major degradation product 2-hydroxymethylbenzonitrile was formed with a maximum amount of 20.1% AR by cleavage of the bridge between the aromatic ring systems.

Adsorption desorption studies indicate that trifloxystrobin can be considered slightly mobile and the major transformation, CGA 321113 can be considered mobile to moderately mobile.

Conclusion:

Based on the above data, trifloxystrobin should be classified as not rapidly degradable for hazard classification.

11.3 Bioaccumulation

Table 32: **Summary of relevant information on bioaccumulation**

Guide-line	Species	Endpoint data	Exposure		Results	Reference
			Design	Duration		
OECD 107	---	Partition coefficient	---	---	log P_{OW} 4.5±0.0094 (25 °C) P_{OW} 32000 ± 680 (25 °C)	Stulz, J. (1997) M-041647-01-1
EPA 165-4 Comparable to OECD 305	*Lepomis macrochirus*	BCF whole fish	Flow through	28 days	BCF: 431 L/kg BCF ($_{\text{lipid normalised 5\%}}$): 370 L/kg	Anonymous (1997e), M-032004-01-1

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

Study 1 - Stulz, J. (1997), M-041647-01-1

The partition coefficient 1-octanol/water of trifloxystrobin was determined in pH 7.51 (average pH of aqueous phase) according to OECD Guideline 107 and GLP (Stulz, J. 1997).

Six amounts of the test substance between 35.2 and 69.6 mg trifloxystrobin dissolved at room temperature in three different volume ratios of octanol and water (20:20; 40:20; 10:20) in duplicates. After shaking for approximately 24 hours, the amount of trifloxystrobin in water and octanol was analysed by HPLC. The results show a P_{OW} of 32000 ± 680 and a corresponding log P_{OW} of 4.5 ± 0.0094 at 25 °C.

Study 2 - Anonymous (1997e), M-032004-01-1

The overall goal of this GLP study was to determine the bioconcentration factor (BCF) of trifloxystrobin for bluegill sunfish *(Lepomis macrochirus)*. The exposure of bluegill to [^{14}C]-trifloxystrobin at nominal concentrations of 0.00016 and 0.0016 mg/L was continuous (flow-through) throughout the establishment of a steady state tissue residue concentration and maintained for 28 days. During the study, 5 fish were removed from each group for total ^{14}C measurement in the edible and viscera tissues at days 1, 3, 7, 10, 14, 16, 21 and 28 of exposure, and at 1, 3, 7, 10 and 14 days after the depuration phase was initiated. Five fish were collected

from the metabolism aquarium on days 21 and 28. These fish were dissected into three portions, edible, viscera and carcass.

Bioconcentration factors (BCF) for each tissue type were calculated using the mean measured steady state exposure water concentration of trifloxystrobin and the mean measured steady state tissue concentrations (based on total [^{14}C]-residues). At both test concentrations, residues of [^{14}C]-trifloxystrobin accumulated within the exposed fish. Equilibrium levels were reached within 3 days in the 0.00016 mg/L group and within 14 days in the 0.0016 mg/L concentration. Steady state BCF for whole fish was calculated to be 280 to 431 L/kg for the two treatments.

Noting the fish lipid content was 5.83% a lipid normalised (5%) BCF is 370 L/kg (Industry communication, 2018).

Within 24 h of being placed in clean water, levels of (^{14}C) in fish had fallen to 69 and 73.4% of final 28 day exposure levels for the 0.0016 and 0.00016 mg/L groups respectively. At the end of the 14-day depuration period, greater than 98% of the accumulated radioactive residue was eliminated from the fish tissue. The respective times for 50 and 90% depuration were given as 0.5 to 2.4 days and 1.5 to 7.8 days.

Summary and discussion of aquatic bioaccumulation
The substance has a log P_{OW} of 4.5 at pH 7.5, which is above the classification criteria of 4. An experiment bioaccumulation study is available that shows that trifloxystrobin does not meet the CLP bioaccumulation criteria of 500.

11.4 Acute Aquatic Hazard

Valid studies relevant for the classification of trifloxystrobin are presented in Table 33.

Eleven degradants were observed in fate studies with CGA 321113 (EE) identified as the major transformation product. Ecotoxicity studies to using CGA 321113 (EE) and other degradants are available and detailed in the RAR. While overall, degradants of trifloxystrobin are not considered more toxic than the parent substance and not considered further for classification.

Table 33: Summary of relevant information on acute aquatic toxicity

Guide-line	Species	Endpoint data	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/L)[1]	
Fish and amphibians							
OECD 203	*Oncorhynchus mykiss*	Mortality	Flow through	96 h	LC_{50}	**0.015 mm**	Anonymous (1997f) M-032048-01-1
OECD 203	*Lepomis macrochirus*	Mortality	Flow through	96 h	LC_{50}	0.054 mm	Anonymous (1997g) M-032068-01-1
OECD 203	*Cyprinodon variegatus*	Mortality	Flow through	96 h	LC_{50}	0.078 mm	Anonymous (1996a) M-032072-01-1
No formal TG	*Xenopus laevis*	Mortality	Flow through	48 h	LC_{50}	0.038 mm	Anonymous (2009) M-358069-01-1
Aquatic invertebrates							
FIFRA 72-2	*Daphnia magna*	Immobilization	Flow through	48 h	EC_{50}	**0.016 mm**	Neumann, C. (1997) M-051484-01-1
FIFRA 72-2	*Daphnia magna*	Mortality	Flow through	48 h	LC_{50}	0.0253 mm	Boeri, R. (1997) M-032084-01-1
EPA 72-2(a)	*Procambarus acutus acutus*	Mortality	Flow through	96 h	LC_{50}	>0.31 mm	Ward, T., (1998) M-052687-01-1
EPA 72-3(b)	*Crassostrea virginica*	Mortality	Flow through	96 h	EC_{50} LC_{50}	0.0349 mm (shell deposition) >0.0748 mm	Boeri, R. (1996) M-032088-01-1
Algae							
OECD 201	*Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*)	Cell number	Static	72 h	E_rC_{50}	**0.0174 mm**	Grade, R. (1995) M-032098-01-1 Recalculation by: Herno, V. (2017) M-032098-01-1

[1] 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

All studies summarized below were conducted according to GLP.

Study 1 - Anonymous (1997f) M-032048-01-1
In a 96-hour flow-through acute toxicity laboratory study, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to the test substance trifloxystrobin (purity: 96.4%). Two replicates of ten fish per concentration and control (blank control and vehicle control with 0.096 mL dimethylformamide/L) in glass aquariums with 15 L water were used for testing. Nominal and mean measured concentrations were 0.004, 0.0072, 0.013, 0.023 and 0.042 mg a.s. /L. and 0.004, 0.0072, 0.0122, 0.0213 and 0.0410 mg a.s. /L (93% – 101% of nominal). Fish were observed for mortality and sublethal symptoms such as abnormal behavioural activity and stress at 2, 24, 48, 72 and 96 hours after test initiation. After 96 hours exposure, mortality occurred in the highest concentrations of 0.0122, 0.0213 and 0.0410 mg a.s. /L with 20, 100 and 100% mortality, respectively. Sublethal effects were observed at concentrations > 0.0072 mg a.s. /L, such as loss of equilibrium, change in the swimming behaviour, in the pigmentation and in the respiratory function, hence, the highest concentration with no sublethal and lethal effects was 0.0072 mg a.s. /L. The LC_{50} (96h) of trifloxystrobin was determined to be 0.015 mg a.s. /L based on mean measured concentrations.

Study 2 - Anonymous (1997g) M-032068-01-1
The test was conducted over a period of 96 hours with *Lepomis macrochirus* in dechlorinated tap water. Fish were exposed to trifloxystrobin (purity: 96.4%). Two replicates, each containing ten fish per concentration and control (blank control and vehicle control with 87 mg dimethylfomamide/L) were exposed under flow-through conditions in one 20 L glass aquarium (with 15 L water) per replicate to nominal test concentrations of 0.017, 0.031, 0.056, 0.10 and 0.18 mg a.s. /L. Mean measured concentrations were 0.015, 0.028, 0.046, 0.076 and 0.15 mg a.s. /L (76% - 91% of nominal). Small particles appeared at the surface of the test solution after 72 h of exposure at concentrations 0.031 and 0.056 mg a.s. /L. At 2, 24, 48, 72 and 96 h, observations of mortality and sublethal symptoms, such as abnormal behavioural activity and stress were made. Sublethal effects were observed after 2-4 h of exposure at concentration 0.076 mg a.s. /L, such as a loss of equilibrium and change in the swimming behaviour, hence, the highest concentration with no sublethal and lethal effects was 0.028 mg a.s. /L. After 96 h exposure, mortality occurred at concentrations of 0.046, 0.076 and 0.15 mg a.s. /L with 5, 100 and 100%, respectively. The LC_{50} (96 h) of trifloxystrobin was determined to be 0.054 mg a.s. /L based on mean measured concentrations.

Study 3 - Anonymous (1996a) M-032072-01-1
The test was conducted over a period of 96 hours with *Cyprinodon variegatus* in natural saltwater adjusted to a salinity of 16 - 17 ppt. Fish were exposed to trifloxystrobin (purity: 95.5%). Two replicates of ten fish per concentration and control (control and solvent control with 0.1 mL dimethylformamide/L) were exposed under flow-through conditions in one 20 L glass aquarium per replicate to nominal test concentrations of 0.039, 0.066, 0.11, 0.18, and 0.30 mg a.s. /L. Analytical determination was performed with samples collected from each replicate test vessel after 0 and 96 hours (HPLC-UV). Mean measured concentrations were 0.0323, 0.0592, 0.0987, 0.166 and 0.259 mg a.s. /L (83% - 92% of nominal). At 0, 24, 48, 72 and 96 h, observations of mortality, and sublethal symptoms were made. 100% survival occurred in the control and 95% survival occurred in the solvent control. No sublethal effects were noted in the controls during the exposure period. Mortality was observed in nominal concentrations of 0.066, 0.11, 0.18 and 0.30 mg a.s. /L (15, 6, 0 and 0 fish were alive at test end, respectively). Sublethal effects were observed in test vessels containing 0.11, 0.18, and 0.30 mg a.s. /L during the test. Exposure of fish to the test substance resulted in a 96 h-LC_{50} of 0.0780 mg a.s. /L, based on mean measured concentrations.

Study 4 - Anonymous (2009) M-358069-01-1
A GLP acute toxicity to *Xenopus laevis* under flow through conditions is available using trifloxystrobin (purity: 99.5%). While the 48 hour study did not follow a dedicated guideline, the test protocol was based on relevant test guidelines such as OECD Test Guideline 203.

Juvenile tadpoles (body length 16.2 ± 0.71 mm) were used in glass aquaria with 7 litres of test media. Study conditions were considered suitable (22-22.4 ºC, 16 hour light photo period, pH 8.1-8.2, dissolved oxygen 85-92%). The exposure range was 9.38, 18.8, 37.5, 75 and 150 µg/L. In addition, a solvent control was included (0.1 mL acetone/L). Three replicates were employed per treatment and control each containing 10 tadpoles. Observations of mortality and sub-lethal effects were conducted at 4, 24 and 48 hours. Analytical measurements were 69 to 96% of nominal.

Based on mean measured concentrations the 48 hour LC_{50} was 38.6 µg a.s. /L equating to 0.038 mg a.s. /L.

11.4.2 Acute (short-term) toxicity to aquatic invertebrates

All studies summarized below were conducted according to GLP.

Study 1 - Neumann, C. (1997) M-051484-01-1
The test was conducted over a period of 48 hours with *Daphnia magna* clone 5 in Elendt M4 medium. Daphnids were exposed to trifloxystrobin (purity: 96.4%). Two replicates with ten daphnids each were applied per concentration and control (blank control and solvent control: 89 mg dimethylformamide/L) and were exposed under flow-through conditions in 400 mL glass vessels (with 250 mL solution renewed every hour by intermittent flow) to nominal test concentrations of 0.0075, 0.015, 0.03, 0.06 and 0.12 mg a.s. /L. Water samples of each concentration were taken at hour 0, 24 and 48 and were analyzed using HPLC with UV detection. Mean measured concentrations were 0.0048, 0.010, 0.023, 0.06 and 0.12 mg a.s. /L. Immobilization or other behavioural changes of the daphnids were recorded after 24 and 48 hours of exposure. Other sublethal effects were also recorded. After 48 hours of exposure, rates of 5, 20, 70 and 100% immobilization were observed at mean measured concentrations of 0.0048, 0.010, 0.023, 0.06 and 0.12 mg a.s. /L, respectively. The estimation of effect values was based on mean measured concentrations according to the Probit-model.

Exposure of daphnids to the test substance resulted in a 48 hour EC_{50} of 0.016 mg a.s. /L, with a 95% confidence interval of 0.012-0.021 mg a.s. /L, based on mean measured concentrations.

Study 2 - Boeri, R., (1997) M-032084-01-1
The test was conducted over a period of 48 hours with *Daphnia magna* in deionized water. Daphnids were exposed to trifloxystrobin (purity: 96.0%). Two replicates with ten daphnids each were applied per concentration and control (blank control and solvent control: 0.1 mL dimethylformamide/L) and were exposed under flow-through conditions to nominal test concentrations of 6.5, 12, 18, 31, and 50 µg a.s./L. Analytical determination was performed with samples collected from each replicate test vessel after 0 and 48 hours (HPLC-UV). Mean measured concentrations were 5.99, 10.7, 18.0, 28.6 and 48.9 µg a.s. /L (89% – 100% of nominal). Lethality is the main endpoint in this study. The numbers of surviving organisms, the occurrence of sublethal effects, and observations of insolubility were determined visually and recorded after 24 and 48 hours. 100% survival occurred in the control and 95% survival occurred in the solvent control. No sublethal effects were noted in the controls during the exposure period. 20, 19, 18, 8 and 0 daphnids survived at mean measured concentrations of 5.99, 10.7, 18.0, 28.6 and 48.9 µg a.s./L. Sublethal effects, observed as immobilized daphnids, were noted in test vessels containing 28.6 and 48.9 µg a.s./L during the test.

Exposure of daphnids to the test substance resulted in a 48 hour-LC_{50} of 25.3 µg a.s./L (equivalent to 0.0253 mg a.s./L), with a 95% confidence interval of 21.8 to 29.4 µg a.s./L, based on mean measured concentrations.

Study 3 - Ward, T., (1998) M-052687-01-1
The test was conducted over a period of 96 hours with *Procambarus acutus acutus* (white river crayfish) in deionized water. Crayfish were exposed to trifloxystrobin (purity: 96.4%). Two replicates with ten crayfish per concentration and control (blank control and solvent control with 0.10 mL dimethylformamide/L) were exposed under flow-through conditions in one 20 L-glass aquarium (with 15 L water) per replicate to nominal test concentrations of 55, 92, 150, 250, and 420 µg a.s. /L (average of 5.5 volume additions per 24 hours in each test vessel). Analytical samples were collected from each exposure vessel at the beginning and end of the

test (HPLC-UV). Mean measured concentrations were 43, 65, 120, 180 and 310 µg a.s. /L (87 to 100% of nominal). The numbers of surviving organisms, the occurrence of sublethal effects, and observations of insolubility were determined visually and recorded initially and after 24, 48, 72, and 96 h. 100% survival occurred in the control and solvent control, and no sublethal effects were noted in the controls during the exposure period. 20, 19, 18, 17 and 17 crayfish survived at mean measured concentrations of 43, 65, 120, 180 and 310 µg a.s. /L.

The 24-, 48-, 72- and 96-h LC_{50} values were reported as greater than the highest tested concentration of test substance > 310 µg a.s./L (equivalent to >0.31 mg a.s./L) based on mean measured concentrations.

Study 4 - Boeri, R. (1996), M-032088-01-1
The test was conducted over a period of 96 hours with *Crassostrea virginica* (Eastern Oyster) in unfiltered, natural seawater. Oysters were exposed to trifloxystrobin (purity: 95.5%). Two replicates with ten oysters each were applied per concentration and control (seawater control and solvent control: 0.1 mL dimethylformamide/L) and were exposed under flow-through conditions in 20 L-glass aquaria to nominal test concentrations of 10, 18, 29, 49, and 80 µg a.s. /L. Analytical determination of test substance concentration was performed with samples collected from each replicate test vessel after 0 and 96 hours (HPLC-UV). Mean measured concentrations were 9.81, 16.8, 28.6, 45.2 and 74.8 µg a.s. /L (92 to 99% of nominal). The numbers of surviving organisms and the occurrence of sublethal effects were determined visually and recorded after 24, 48, 72 and 96 hours. No mortality occurred in the control and in the solvent control. No sublethal effects were noted in any test vessel during the exposure period. One oyster died in the highest test concentration.

Exposure of eastern oysters to the test substance resulted in a 96 hour-EC_{50} for shell growth of 34.9 µg a.s./L (equivalent to 0.0349 mg a.s./L), with a 95% confidence interval of 19.7 to 62.0 µg a.s./L, based on mean measured concentrations. Given one oyster died at the highest concentration, the 96 hour-LC_{50} is > 74.8 µg a.s. /L (equivalent to >0.0748 mg a.s. /L).

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

The studies summarized below were conducted according to GLP.

Study 1 - Grade, R. (1995), M-032098-01-1 and recalculation - Herno, V. (2017), M-582093-01-1
The aim of the study following OECD Test Guideline 201 (1984), was to assess the 72 hours toxicity of trifloxystrobin (purity: 96.4%) to green algae, *Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*), expressed as inhibition of algal growth, under static test conditions. Three replicates for each test concentration (nominal 0.0020, 0.0044, 0.0096, 0.021, 0.046, 0.10 and 0.22 mg a.s. /L) and six for the control and solvent control (0.0088 mg TWEEN 80/L) were applied.

Samples of test solutions were taken immediately before exposure and after 72 hours exposure. All samples were analysed using HPLC with UV-detection. Arithmetic mean measured concentrations were 0.00104, 0.00192, 0.00238, 0.0159, 0.0204, 0.0359 and 0.0608 mg a.s./L. Geometric mean measured concentrations were 0.00103, 0.00192, 0.00237, 0.0158, 0.0201, 0.0357 and 0.0608 mg a.s./L.

Initial cell density was 9900 cells/mL. Cell densities were measured at 24, 48 and 72 hours exposure. The biomass in the blank and solvent control increased by a factor of 143 and 129 during the test indicating test validity criteria were met.

The E_rC_{50} (0-72h) was 0.016 mg a.s. /L based on arithmetic mean measured concentrations. The NOEC was determined to be 0.00192 mg a.s. /L based on both arithmetic and geometric mean measured concentrations.

The endpoints were re-calculated on the basis of geomean measured concentrations, in addition EC_{10} and EC_{20} values were provided. The recalculated endpoints, based on geometric mean measured concentrations were:

- E_rC_{50} of 0.0174 mg a.s./L
- E_rC_{10} is 0.0025 mg a.s./L

Study 2 - Ward, T.J. et al. (1996), M-032662-01-1

The static test with *Lemna gibba* was performed according to FIFRA guideline 123-2 over 14 days at a temperature of 25 ± 2°C. Comparison of study data with current test guideline criteria (OECD Test Guideline 221 [2006]) identified that study controls were not valid at either 7 or 14 days (Herno, 2018). On this basis, the study is not considered reliable.

11.4.4 Acute (short-term) toxicity to other aquatic organisms

No other acute toxicity test relevant for classification purposes, is available on trifloxystrobin.

11.5 Long-term aquatic hazard

Table 34: Summary of relevant information on chronic aquatic toxicity

Guide-line	Species	Endpoint data	Exposure		Results		Reference
			Design	Duration	Endpoint[1]	Toxicity (mg/L)[2]	
Fish							
EPA 72-4(a)	*Oncorhynchus mykiss*	Survival and development	flow through	ELS, 95 days	NOEC EC_{10}	0.0043 mm (time to swim-up) **0.0075 mm (**survival at the end of the test)	Anonymous (1997h) M-032080-02-1
Aquatic invertebrates							
EPA 72-4(b)	*Daphnia magna*	Reproduction	Flow through	21 days	NOEC EC_{10}	0.00276 mm **0.00328 mm**	Boeri, R. (1996) M-032097-01-1 recalculation by Herno, V. (2017) M-582256-01-1
Algae							
OECD 201	*Desmodesmus subspicatus (formerly Scenedesmus subspicatus)*	Cell number	Static	72 h	NOEC E_rC_{10}	0.00192 mm **0.0025 mm**	Grade, R. (1995) M-032098-01-1 recalculation by Herno, V. (2017) M-582093-01-1

[1] Both NOEC and EC_{10} are presented in this table if available, however preference is given to EC_{10} according to the Guidance on the Application of the CLP Criteria Version 5.0 – July 2017

[2] Endpoints in bold are the critical endpoints for that organism group. The letters to the right of the numbers refer to what concen-tration of the active substance the endpoint is based on: n = nominal; mm = mean measured; im = initial measured

11.5.1 Chronic toxicity to fish

The study summarized below was conducted according to GLP.

Study 1 - Anonymous (1997h) M-032080-02-1

The aim of this early life stage toxicity test was to establish chronic toxicity levels of trifloxystrobin (purity: 96.4%) using the most critical and sensitive life stage of the whole life cycle of rainbow trout (*Oncorhynchus mykiss*), in a flow-through system. Four hours after fertilization, 42 embryos were transferred into each replicate egg incubator cup. In addition, 42 fertilized eggs were placed in additional incubation cups in the controls for determining viability (fertilization). 20 L-glass aquaria with 15 L water were used as test vessels, each with 42 eggs, three replicates per treatment and control/solvent control. The nominal test concentrations were: 0.00069, 0.0012, 0.0022, 0.0040, 0.0072, 0.013 mg a.s./L. The overall arithmetic mean measured concentrations for each test level, averaged from the measured concentrations of trifloxystrobin at the beginning, at weekly intervals and at the end of the exposure period were 0.00093, 0.0014, 0.0025, 0.0043, 0.0077 and 0.015 mg a.s./L. In addition to the test item treatments, a blank control (dechlorinated tap water) and a solvent control (83.6 mg dimethylformamide/L) were established. Egg mortality was recorded on working days and dead eggs were removed to prevent fungal growth. Eggs without visible neural keel were removed on day 19. At test termination on study day 95 (60 days post-hatch), all surviving fish were measured for total length, and weighted. Based on the transient effects on survival and time to hatch, the no observed effect concentration for fish exposed for 95 days to trifloxystrobin is 0.0077 mg a.s./L (based on mean measured concentrations). However, the statistically significant delay in time to swim up at 0.0077 mg a.s./L, although reported to be transient, is considered to be treatment related. On this basis it was considered that the NOEC is 0.0043 mg a.s./L. The EC_{10} and EC_{20} for survival at the end of the test are 0.0075 and 0.0079 mg a.s./L, respectively (mean measured).

11.5.2 Chronic toxicity to aquatic invertebrates

The study summarized below was conducted according to GLP.

Study 1 - Boeri, R. (1996) M-032097-01-1 and recalculation - Herno, V. (2017) M-582255-01-1

The aim of the study was to establish chronic toxicity levels of trifloxystrobin (purity: 96.4%) to the freshwater invertebrate *Daphnia magna* in a 21-days exposure test, under flow-through conditions. Daphnids were exposed in 1 L glass vessels. The solvent control contained 0.10 mL dimethylformamide/L. The nominal test concentrations were 0.0032, 0.0065, 0.013, 0.025 and 0.050 mg a.s./L. Analytical determination of trifloxystrobin concentrations was performed with samples collected from each test vessel on days 0, 7, 14, and 21 (HPLC UV). Mean measured concentrations were 0.00276, 0.00598, 0.0120, 0.025 and 0.0506 mg a.s./L. Investigated endpoints were survival of first generation daphnids (on day 21), sublethal effects as immobilization, changes in behaviour or appearance (daily), the time to first brood, the number of young per female (daily from onset of reproduction), and the length and the dry weight of surviving daphnids (on day 21). 5% of parental daphnids in both controls died during the test. The mean number of living offspring produced per control female was 62 in the water control and 57 in the solvent control. Survival of the F0 generation was statistically significantly decreased at concentrations of 0.012 mg a.s./L and above (all daphnids exposed to 0.025 and 0.0506 mg a.s./L were dead prior to day 7). The same pattern was noted on day to first brood. For the average number of young per surviving adult, average dry weight of adults and average length of adults at day 21, statistically signicantly decreases were noted at concentrations of 0.00598 mg a.s./L and above. Sublethal effects, other than visually observed size differences, or immobilization of offspring, were not observed at any time during the test. The 21-day NOEC was 0.00276 mg a.s./L (mean measured), based on mean number of young per surviving *Daphnia*, mean dry weight and mean length. The lowest EC_{10} and EC_{20} relate to mean dry weight in the F1 generation and are 0.00328 and 0.00459 mg a.s./L, respectively.

11.5.3 Chronic toxicity to algae or other aquatic plants

Please refer to chapter 11.4.3 Acute (short-term) toxicity to algae or other aquatic plants.

11.5.4 Chronic toxicity to other aquatic organisms – additional information

The study summarized below was conducted according to GLP.

Study 1 - Grade, R. (1998) M-033988-01-1 and recalculation - Herno, V. (2017) M-582256-01-1
The toxicity of trifloxystrobin (purity: 95.6%) to the sediment-dwelling larvae of the midge *Chironomus riparius* was assessed using a static water-sediment system in 28-day study. The system comprised units of 1 litre glass beakers containing about 1.5 cm of artificial sediment and a water column of 8 cm at the start. Following a range finding study, first instar midge larvae (2-3 days old) were exposed to 6 nominal aqueous concentrations of trifloxystrobin of 0.0125, 0.025, 0.05, 0.1, 0.2 and 0.4 mg a.s./L. In addition there were three blank control and three vehicle (DMF) controls.

Analytical determination of trifloxystrobin and of its main metabolite in sediment was performed with samples collected from each test vessel on days 0, 7, 14, and 28. All samples were analyzed using HPLC with UV-detection. The actual measured concentrations of trifloxystrobin in the water phase were 0.009, 0.021, 0.046, 0.101, 0.212 and 0.416 mg a.s./L at day 0 (1-3 h after application). At the end of the test (day 28) levels of trifloxystrobin in the water phase were below the limit of detection (stated to be 0.0024 mg a.s./L) in all test concentrations. Over the study period the degradand CGA 32113 was observed and increased to 0.004, 0.012, 0.025, 0.056, 0.12 and 0.2 mg/l by day 28. Analysis of sediment confirmed the test item and degradant CGA 32113. Sediment from the two highest test concentrations was analysed on days 0, 7 and 28. At the nominal concentration of 0.2 mg trifloxystrobin/l, the measured concentrations of trifloxystrobin plus CGA 321113 in the sediment (including interstitial water) were 0.10, 0.22 and 0.23 mg/kg sediment (wet) on days 0, 7 and 28 respectively. At the nominal concentration of 0.4 mg trifloxystrobin/l the measured concentrations of trifloxystrobin plus CGA 321113 in the sediment (including interstitial water) were 0.17, 0.79 and 0.36 mg/kg sediment (wet) on days 0, 7 and 28 respectively.

Each test vessel contained 20 larvae, and a total of 3 replicates per test concentration. Visual assessments (behaviour, mortalities, emergence) were made daily. The number, time and sex of emerged adults was recorded. Statistically significant effects were observed at the highest treatment of 0.4 mg a.s./L for development rate and emergence rate. The final mean percentage emergence figures were 86.6, 81.6, 81.6, 81.6, 80, 71.6 and 60 in the blank control, vehicle control 0.025, 0.05, 0.10, 0.2 and 0.4 mg a.s./L groups respectively.

The study NOEC was 0.2 mg a.s./L based on nominal concentrations. The EC_{10} and EC_{20} for emergence rate were 0.14 and 0.32 mg a.s./L based on initial measured concentrations. The NOEC for emergence rate and development rate is 0.21 mg a.s./L based on initial measured concentrations. The decline in aqueous phase concentrations and observed partitioning makes interpretation difficult as it is unclear if a contribution of the toxicity in this study was due to sediment contact/ingestion. Therefore it is not possible to use the quoted study endpoints for hazard classification.

11.6 Comparison with the CLP criteria

11.6.1 Acute aquatic hazard

Acute toxicity data on trifloxystrobin are available for fish, invertebrates, algae and aquatic plants. All trophic groups show similar sensitivity to the substance with lowest endpoints in the range of 0.015 to 0.0174 mg/L. Therefore trifloxystrobin should be classified as Aquatic Acute 1 with an acute M-factor of 10 based on acute endpoints in the range 0.01 to 0.1 mg/L.

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

Evolved CO_2 from non-radiolabelled trifloxystrobin dosed vessels was indistinguishable from that of control vessels. Trifloxystrobin is therefore classified as ‘not readily biodegradable’.

Hydrolytic degradation of trifloxystrobin (EE) in sterile aqueous buffer solutions in the dark in the laboratory is strongly dependent on the temperature and the pH value. The longest half-life for CGA 321113 within the pH range 4 to 9 was in excess of 1000 days when adjusted via calculation to 12 °C.

Under aerobic conditions, trifloxystrobin rapidly hydrolyses (DT_{50} ~ 3.3 days at 12 °C) to form metabolite CGA 321113. Very little mineralisation occurred during the study. Sterilised test vessels showed similar degradation of trifloxystrobin/formation of CGA 321113 as non-sterilised test systems, implying that the degradation of trifloxystrobin in aerobic surface waters is an abiotic process.

In water –sediment systems, trifloxystrobin (EE) underwent rapidl primary degradation to the major degradation CGA 321113 (EE), non-extractable residues and low levels of CO_2. Data from the two aerobic sediment-water studies combined and statistically examined yielded the following DT_{50} values for trifloxystrobin and CGA 321113 at 12 °C. For trifloxystrobin DT_{50} values were calculated as 1.44 days, 4.65 days and 3.21 days for water, sediment and total system phases respectively reflecting primary degradation. For CGA 321113, DT_{50} values were calculated as 398 days, >1000 days and 736 days for water, sediment and total system phases respectively. Under photolytic conditions in the laboratory in sterile buffers at pH 5 and pH 7 and in sterile natural water, trifloxystrobin (EE) was rapidly degraded (DT50 ≤ 1.7 days) by E/Z isomerization (in this summary referred to as "photodegradation products").

Overall, trifloxystrobin is not considered to be ultimately degraded in the aquatic environment to a level > 70 % within a 28-day period.

Ecotoxicity data (presented in the DAR) for primary degradation products indicate that major degradant CGA 321113 and minor degradant CGA 357261 may fulfil the criteria for classification as hazardous to the aquatic environment. Therefore it cannot be considered that trifloxystrobin undergoes primary degradation to products that do not fulfil the criteria for classification as hazardous to the aquatic environment.

Overall, according to the CLP criteria, trifloxystrobin is considered not rapidly degradable.

Adsorption desorption studies indicate that trifloxystrobin can be considered slightly mobile and the major transformation, CGA 321113 can be considered mobile to moderately mobile.

As available BCFs are < 500, therefore trifloxystrobin does not meet CLP criteria for bioaccumulation.

Chronic toxicity data for trifloxystrobin are available on fish, invertebrates, algae and aquatic plants. The lowest endpoint is 0.0025 mg/L for green algae. Therefore, trifloxystrobin should be classified as Aquatic Chronic 1 with a chronic M-factor of 10 based on chronic endpoints in the range 0.001 to 0.01 mg/L for a not rapidly degradable substance.

11.7 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Aquatic Acute 1; H400: Very toxic to aquatic life

Acute M-factor = 10

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

Chronic M-factor = 10

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Summary

Trifloxystrobin has an existing entry in Annex VI of the CLP regulation as Aquatic Acute 1; H400 and Aquatic Chronic 1; H410. This proposal seeks to confirm the existing entry and assign M-factors.

The proposal from the DS concluded that trifloxystrobin is not rapidly degradable for hazard classification based on the absence of degradation in a valid OECD TG 301B study. Under aerobic conditions, trifloxystrobin was also shown to hydrolyse rapidly.

Trifloxystrobin has a lipid normalised (5 %) bioconcentration factor (BCF) of 370 L/kg and a Log P_{OW} of 4.5 at 25 °C, indicating a low potential for bioaccumulation.

Valid aquatic acute- and long-term studies for all trophic levels were available. All studies presented in the CLH report were conducted according to GLP. Regarding the acute tests included in the report, all trophic groups showed similar sensitivity to the test substance, with the lowest endpoints in the range of 0.015 to 0.0174 mg/L. Based on these endpoints, the DS concluded that trifloxystrobin should be classified as Aquatic Acute 1, with an acute M-factor of 10 as the data falls in the range 0.01 to 0.1 mg/L. However, during the public consultation, a comment was received relating to the presence of a study, evaluated during the pesticidal active-substance renewal program for trifloxystrobin, on *Mysidopsis bahia*. As the study was deemed valid by the DS and derived a more conservative toxicity value (96h LC_{50} of 0.00862 mg/L) this value was used by the DS to change the proposed acute M-factor from 10 to 100.

Chronic toxicity data for trifloxystrobin were available for fish, invertebrates, algae and aquatic plants. The lowest endpoint is a 72h E_rC_{10} = 0.0025 mg/L for green algae (*Desmodesmus subspicatus*). Therefore, as trifloxystrobin is considered as not rapidly degradable, the DS proposed to classify the trifloxystrobin as Aquatic Chronic 1 with an M-factor of 10.

Degradation

Two valid aqueous hydrolysis studies performed under GLP were available. Both studies illustrated that trifloxystrobin rapidly hydrolyses to form the metabolite CGA 321113 and that the hydrolytic degradation of trifloxystrobin is pH-dependent. Rates of degradation increased as the pH increased from pH 5 to pH 13, where the fastest hydrolytic degradation rates were observed.

Four valid photolysis studies performed under GLP are available. Under photolytic conditions in the laboratory in sterile buffers at pH 5 and pH 7 and in sterile natural water, both trifloxystrobin and its major metabolite CGA 321113 were shown to rapidly degrade.

A valid, GLP-compliant study on rapid degradability is available, according to OECD TG 301B (1992). Evolved CO_2 concentrations from the trifloxystrobin (CGA 279202 tech.) samples were identical to that of the untreated inoculum at 28 days and thus no rapid degradability could be concluded.

In a valid, GLP-compliant, water simulation study, performed following the OECD TG 309, trifloxystrobin hydrolysed rapidly to form the metabolite CGA 321113. Degradation in surface waters appears to occur only via abiotic processes. The DT_{50} was calculated to be 3.4 and 3.3 days at 12 °C for high and low concentrations, respectively. Two valid, GLP-compliant aerobic sediment/water studies were available. For trifloxystrobin, the DT_{50} (12 °C) values were calculated to be around 2.1 days, 6.3-7.4 days and 5.3-6.1 days for river water, river sediment and total river system, respectively. For the metabolite CGA 321113, DT_{50} (12 °C) values were calculated as 560-588 days, 872 to > 1 000 days and 7 210 to > 1 000 days for river water, river sediment and total river system, respectively. A third study is available that offers a re-evaluation of the validated data from previous studies. The DT_{50} (12 °C) values for trifloxystrobin were calculated as 1.44 days, 4.65 days and 3.21 days for water, sediment and

total system compartments, respectively. For the metabolite CGA 321113, DT_{50} (12 °C) the values were calculated as 398 days, > 1 000 days and 736 days for water, sediment, and total system compartments, respectively.

Bioaccumulation

A GLP-study is available that determined the BCF of trifloxystrobin for bluegill sunfish (*Lepomis macrochirus*). The study was performed following the EPA 165-4 guideline and was considered comparable to OECD TG 305. Steady state BCF for whole fish was calculated to be 431 L/kg and the corresponding lipid normalised (5 %) BCF was reported to be 370 L/kg.

In a GLP-study performed according to OECD TG 107, the 1-octanol/water partition coefficient of trifloxystrobin was determined in pH 7.51 (average pH of aqueous phase) as Log P_{OW} of 4.5 ± 0.0094 at 25 °C.

Therefore, the DS concluded that trifloxystrobin has low bioaccumulation potential.

Acute Aquatic Toxicity

There are several, GLP-compliant, acute studies for all trophic levels available. Four studies are available for fish and aquatic invertebrates respectively and one study for algae. The data for all the studies are summarised in the table below. Trifloxystrobin metabolites as reported by the DS are not considered more toxic than the parent substance and thus they are not considered further for classification purposes.

***Table**: Summary of the relevant acute toxicity data on fish, aquatic invertebrates and algae/aquatic plants (key data are highlighted in bold)*

Guideline	Species	Endpoint data	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/L)	
Fish and amphibians							
OECD TG 203	*Oncorhynchus mykiss*	Mortality	Flow through	96h	LC_{50}	**0.015 mm**	Anonymous (1997f) M-032048-01-1
OECD TG 203	*Lepomis macrochirus*	Mortality	Flow through	96h	LC_{50}	0.054 mm	Anonymous (1997g) M-032068-01-1
OECD TG 203	*Cyprinodon variegatus*	Mortality	Flow through	96h	LC_{50}	0.078 mm	Anonymous (1996a) M-032072-01-1
No formal TG	*Xenopus laevis*	Mortality	Flow through	48h	LC_{50}	0.038 mm	Anonymous (2009) M-358069-01-1
Aquatic invertebrates							
FIFRA 72-2	*Daphnia magna*	Immobilisation	Flow through	48h	EC_{50}	**0.016 mm**	Neumann (1997) M-051484-01-1
FIFRA 72-2	*Daphnia magna*	Mortality	Flow through	48h	LC_{50}	0.0253 mm	Boeri (1997) M-032084-01-1
EPA 72-2(a)	*Procambarus acutus*	Mortality	Flow through	96h	LC_{50}	>0.31 mm	Ward (1998) M-052687-01-1
EPA 72-3(b)	*Crassostrea virginica*	Mortality	Flow through	96h	EC_{50} LC_{50}	0.0349 mm (shell deposition) > 0.0748 mm	Boeri (1996) M-032088-01-1

Algae							
OECD TG 201	*Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*)	Cell number	Static	72h	E_rC_{50}	**0.0174 mm**	Grade (1995) M-032098-01-1 Recalculation by: Herno (2017) M-032098-01-1

mm = mean measured

All trophic groups showed similar sensitivity to the substance. The lowest endpoints for fish, aquatic invertebrates and algae, based on mean measured concentrations, were 96h 0.015 mg/L, 48h 0.016 mg/L and 72h 0.0174 mg/L, respectively. Based on the above-mentioned endpoints, the DS concluded that trifloxystrobin should be classified as Aquatic Acute 1 with an acute M-factor of 10 based on acute endpoints, which fall in the range 0.01 to 0.1 mg/L.

However, during the public consultation, a comment was received relating to the presence of a study, evaluated during the pesticidal active-substance renewal program for trifloxystrobin, on *Mysidopsis bahia*. As the study was deemed valid by the DS and derived a more conservative toxicity value (96h LC_{50} of 0.00862 mg/L), this value was used by the DS to change the proposed acute M-factor from 10 to 100. More details on the study can be found in the section below that summarises the comments received during the public consultation.

Chronic Aquatic Toxicity

One long-term study for each trophic level is available. The data for all the studies are summarised in the table below. As mentioned above, trifloxystrobin metabolites as reported by the DS are not considered more toxic than the parent substance and, thus, they are not considered further for classification purposes.

***Table**: Summary of the relevant chronic toxicity data on fish, aquatic invertebrates and algae/aquatic plants (key data are highlighted in bold).*

Guideline	**Species**	**Endpoint data**		**Exposure**		**Results Toxicity (mg/L)**	**Reference**
		Effects endpoint	**Design**	**Duration**	**Endpoint**		
Fish							
EPA 72-4(a)	*Oncorhynchus mykiss*	Survival and development	Flow through	ELS, 95d	NOEC EC_{10}	0.0043 mm (time to swim-up) 0.0075 mm (survival at the end of the test)	Anonymous (1997h) M-032080-02-1
Aquatic invertebrates							
EPA 72-4(b)	*Daphnia magna*	Reproduction	Flow through	21d	NOEC EC_{10}	0.00276 mm 0.00328 mm	Boeri (1996) M-032097-01-1 recalculation by Herno (2017) M-582256-01-1

Algae							
OECD TG 201	*Desmodesmus subspicatus (formerly Scenedesmus subspicatus)*	Cell number	Static	72h	NOEC E_rC_{10}	0.00192 mm **0.0025 mm**	Grade (1995) M-032098-01-1 recalculation by Herno (2017) M-582093-01-1

mm = mean measured

Based on trifloxystrobin being non-rapidly degradable and based on the toxicity value for algae (*Desmodesmus subspicatus*), the DS proposed that trifloxystrobin should be classified as Aquatic Chronic 1 with an acute M-factor of 10, based on the chronic endpoint of 72h E_rC_{10} = 0.0025 mg/L, which falls in the range 0.001 to 0.01 mg/L.

Comments received during public consultation

One MSCA commented and agreed with the initial environmental classification as it was proposed by the DS. A second MSCA also agreed with the proposed environmental classification but drew attention to an additional acute toxicity study (Boeri, 1996) that would lead to an acute M-factor of 100 instead of the proposed value of 10. As mentioned by the commenting MS, during the Annex I renewal process for trifloxystrobin, the Rapporteur Member State (RMS) concluded that a reliable toxicity endpoint could not be derived for this study, therefore the RMS considered that it was not suitable for use in the risk assessment (page 113 of dRAR Vol 3 B.9 (AS), July 2017). However, upon further consideration from the DS, the study (Boeri, 1996) was concluded to be reliable for the purpose of hazard classification. The DS based its decision to consider the study for classification purposes on the fact that the study was conducted according to GLP, follows the US FIFRA guideline 72-3 with the validity criteria being met. The study reported a 96h LC_{50} of 0.00862 mg/L (based on mean measured concentrations).

The DS agreed that the additional acute study could be used for classification purposes. The use of these data would change the M-factor of the proposed acute classification from 10 to 100.

Assessment and comparison with the classification criteria

Degradation

Trifloxystrobin was shown to degrade rapidly through hydrolysis and photolysis. No significant concentrations of CO_2 were measured in a valid, GLP-compliant study on rapid degradability study (OECD TG 301B (1992)) during 28 days of incubation. Trifloxystrobin does not fulfil the criterion for carbon dioxide generation of 60 % of the theoretical maximum. Consequently, RAC agrees that trifloxystrobin is not rapidly degradable for the purpose of classification and labelling. It should be noted that the main metabolite (CGA 321113) is not considered more toxic than the parent molecule and thus is not considered further for classification purposes.

Bioaccumulation

Trifloxystrobin has a reliable, lipid normalised BCF of 370 L/kg, which is below the CLP criterion of ≥ 500 L/Kg. Although the Log P_{ow} is above the CLP criterion for Log P_{ow} of ≥ 4, the high

quality BCF is given preference. Consequently, RAC agrees with the DS that trifloxystrobin is not bioaccumulative.

Aquatic Toxicity

Acute toxicity

The critical acute endpoint is a 96h LC_{50} of 0.00862 mg/L for aquatic invertebrates (*Mysidopsis bahia*) from the Boeri (1996) study. This study, as mentioned by the DS, was performed under GLP and following US FIFRA guideline 72-3 with the validity criteria being met. Small derogations from the above-mentioned guideline on the testing temperature and the testing photoperiod exist, although RAC considers this study to be reliable and relevant for hazard classification.

This value is below the 1 mg/L criterion and thus the classification of trifloxystrobin as Aquatic Acute 1 should be retained. Also, the value is in the range of $0{,}001 < L(E)C_{50} \leq 0{,}01$ which justifies an acute M-factor of 100. Thus, RAC agrees that trifloxystrobin should be classified as Aquatic Acute 1 with an M-factor of 100.

Chronic toxicity

The critical chronic endpoint is the 72h E_rC_{10} = 0.0025 mg/L for algae (*Desmodesmus subspicatus*). This value is in the range of below 0.1 mg/L which is the classification threshold for Aquatic Chronic 1 for not rapidly degradable substances, and justifies a chronic M-factor of 10 ($0.001 < NOEC \leq 0.01$ mg/L). Thus, RAC agrees that trifloxystrobin should be classified as Aquatic Chronic 1 with an M-factor of 10.

Conclusion on classification

Trifloxystrobin is considered not rapidly biodegradable and is not bioaccumulative.

RAC agrees with the DS that Trifloxystrobin warrants **classification as Aquatic Acute 1; H400 with an M-factor of 100, and Aquatic Chronic 1; H410 with an M-factor of 10.**

12 EVALUATION OF ADDITIONAL HAZARDS

Not applicable to this CLH submission. There is no requirement to consider the classification for additional hazards.

13 ADDITIONAL LABELLING

Not applicable.

14 REFERENCES

Angly, H.; Report on explosive properties; Institute of Safety and Security, Basel, Switzerland; Report No.: 97.4029.EXP; Document No.: M-041830-01-1; 1997-08-26; Pages: 6

Angly, H.; Report on flammability of solids; Institute of Safety and Security, Basel, Switzerland; Report No.: 97.4029.FLS; Document No.: M-041812-01-1; 1997-08-26; Pages: 4

Angly, H.; Report on oxidizing properties of solids; Institute of Safety and Security, Basel, Switzerland; Report No.: 97.4029.OXP; Document No.: M-043079-01-1; 1997-08-26; Pages: 4

Angly, H.; Report on relative self-ignition temperature for solids; Institute of Safety and Security, Basel, Switzerland; Report No.: 97.4029.AFS; Document No.: M-041821-01-1; 1997-08-26; Pages: 5

Angly, H.; Report on screening test for thermal stability and stability in air; Institute of Safety and Security, Basel, Switzerland; Report No.: 97.4029.TSA; Document No.: M-041479-01-1; 1997-08-26; Pages: 6

Anonymous (1994); CGA 279202 tech. - 28-days range finding study in rats (administration in food); M-040074-01-1

Anonymous (1996a); Acute toxicity of CGA 279202 to the sheepshead minnow, *Cyprinodon variegatus;* Report No.: 672-CG; Document No.: M-032072-01-1; 1996-11-06; Pages:30

Anonymous (1997a); The metabolism of [trifluormethyl-phenyl(U)-14C] CGA 279202 after multiple oral administration to lactating goats; M-034501-01-1

Anonymous (1997b); The metabolism of [glyoxyl-phenyl-(U)-14C] CGA 279202 after multiple oral administration to lactating goats; M-034517-01-1

Anonymous (1997c); CGA 279202 - Magnitude of the residues in meat and milk resulting from the feeding of three levels to dairy cattle; M-038221-01-1

Anonymous (1997d); CGA 279202 tech. - 3-month oral toxicity study in rats (administration in food); M-040135-01-1

Anonymous (1997e); [Phenyl(A)-U-14C]-CGA-279202 - Flow-through bioconcentration and metabolism study with bluegill sunfish (*Lepomis macrochirus*); Report No.: 96-8-6608; Document No.: M-032004-01-1; 1997-09-29; Pages: 552

Anonymous (1997f); Acute toxicity test of CGA 279202 to rainbow trout (Oncorhynchus mykiss) in the flow-through system; M-032048-01-1

Anonymous (1998b); Absorption, distribution amd excretion of [trifluormethyl-phenyl-(U)-14C] and [glyoxyl-phenyl-(U)-14C] CGA 279202 in the rat (extension); Report No.: 20/97; Document No.: M-136744-01-1; 1998-01-08; Pages: 80

Anonymous (1998a); Absorption, distribution and excretion of (glyoxyl-phenyl-U-14C) and (trifluormethyl-phenyl-U-14C) CGA 279202 in the rat; Report No.: 13/96; Document No.: M-136746-01-1; 1998-01-28; Pages: 131

Anonymous (1998c); The metabolism of [glyoxyl-phenyl-(U)-14C] and [trifluormethyl-phenyl-(U)-14C] CGA 279202 in the rat; M-136745-01-1

Anonymous (1998d); CGA 279202 tech. - 24-month carcinogenicity and chronic toxicity study in rats; M-040512-02-1

Anonymous (1997g); Acute toxicity of CGA 279202 to bluegill (*Lepomis macrochirus*) under flow-through conditions; Report No.: 963541; Document No.: M-032068-01-1; 1997-09-22; Pages: 63

Anonymous (2009); Acute toxicity of trifloxystrobin technical to *Xenopus laevis* under flow-through conditions; Report No.: EBTFY003; Document No.: M-358069-01-1; 2009-10-27; Pages: 46

Anonymous (1999b); CGA 279202 technical - Rabbit oral teratogenicity; Report No.: 943043; Document No.: M-039377-03-1; 1999-12-20; Pages: 420

Anonymous (1997h); Early life-stage toxicity of CGA 279202 to rainbow trout (*Oncorhynchus mykiss*) using newly fertilized "green" eggs in a flow-through system; Report No.: 943530; Document No.: M-032080-02-1; 1997-11-07; Pages: 112

Anonymous (1996b); YRC 2894 - Developmental toxicity study in rabbits after oral administration; Report No.: 24709; Document No.: M-000780-01-1; 1996-01-26; Pages: 404

Anonymous (1999a); CGA 279202 technical - Rat oral teratogenicity; M-039420-02-1

Anonymous (2001); CGA 279202 Technical - Rat dietary two-generation reproduction study; M-039264-02-1

Boeri, R. L.; Magazu, J. P.; Ward, T. J.; Acute flow-through mollusc shell deposition test with CGA 279202; Wilbury Laboratories, Inc., Marblehead, MA, USA; Report No.: 674-CG; Document No.: M-032088-01-1; 1996-12-20; Pages: 31

Boeri, R. L.; Magazu, J. P.; Ward, T. J.; Acute toxicity of CGA 279202 to the daphnid, *Daphnia magna*; Wilbury Laboratories, Inc., Marblehead, MA, USA; Report No.: 1116-CG; Document No.: M-032084-01-1; 1997-03-27; Pages: 30

Boeri, R. L.; Magazu, J. P.; Ward, T. J.; Chronic toxicity of CGA 279202 to the daphnid, *Daphnia magna*; Wilbury Laboratories, Inc., Marblehead, MA, USA; Report No.: 1117-CG; Document No.: M-032097-01-1; 1996-08-26; Pages: 44

Burkhard, N.; Henry's law constant - CGA 279202; Novartis Crop Protection AG, Basel, Switzerland; Report No.: MO-01-003756; Document No.: M-041515-01-1; 1997-09-01; Pages: 1

Chahoud, I.; Buschmann, J.; Clarkm R.; Druga, A.; Falke, H.; Faqi, A.; Hansen, E.; Heinrich-Hirsch, B.; Hellwig, J.; Lingk, W.; Parkinson, M.; Paumgartten, F. J. R.; Pfeil, R.; Platzek, T.; Scialli, A. R.; Seed, J.; Stahlmann, R. et al; Classification in terms in developmental toxicology: Need for harmonisation; Freie Universitaet Berlin, Berlin, Germany; Institute for Toxicology and Aerosol Research, Hannover, Germany; Ruth Clark Associates Ltd, United Kingdom; Institute for Drug Research Ltd., Budapest, Hungary; Board of the Authorisation of Pesticides, ...; Report No.: M-610139-01-1; Document No.: M-610139-01-1; 1999-12-31; Pages: 6

Das, R.; Report on boiling point / boiling range - CGA 279202; Ciba-Geigy Limited, Muenchwilen, Switzerland; Report No.: 46881; Document No.: M-041467-01-1; 1996-12-12; Pages: 4

Das, R.; Report on general physico-chemical properties, pure a.i. (aspect, colour, odour) - CGA 279202; Ciba-Geigy Limited, Muenchwilen, Switzerland; Report No.: 46887; Document No.: M-041523-01-1; 1996-11-27; Pages: 2

Das, R.; Report on general physico-chemical properties, technical grade a.i. (aspect, colour, odour) - CGA 279202; Novartis Crop Protection Muenchwilen AG, Muenchwilen, Switzerland; Report No.: 53274; Document No.: M-041530-01-1; 1997-08-25; Pages: 2

Das, R.; Report on melting point / melting range - CGA 279202; Ciba-Geigy Limited, Muenchwilen, Switzerland; Report No.: 46880; Document No.: M-041431-01-1; 1996-11-27; Pages: 3

ECHA (2017), Guidance for Information Requirements and Chemical Safety Assessment. Chapter R.7b: Endpoint specific guidance. Version 4.0 (Public). European Chemicals Agency, Helsinki.

ECHA (2017), Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.11: PBT/vPvB Assessment. Version 3, Technical Report ECHA-17-G-07-EN. European Chemicals Agency, Helsinki.

Fahrbach, M.; [Benzeneacetic-phenyl-UL-14C]trifloxystrobin: Aerobic mineralization in surface water; Harlan Laboratories Ltd., Itingen, Switzerland; Report No.: D60632; Document No.: M-449602-01-1; 2013-03-08; Pages: 86

Food & Agriculture Organization of the United Nations (FAO; 2000), Appendix 2. Parameters of pesticides that influence processes in the soil. In FAO Information Division Editorial Group (Ed.), *Pesticide Disposal Series 8. Assessing Soil Contamination. A Reference Manual*. Rome: Accessed July 10, 2009)

Fueldner, H.; Report on density of solids - CGA 279202; Ciba-Geigy Limited, Basel, Switzerland; Report No.: PP-96/63P.DES; Document No.: M-041496-01-1; 1997-02-07; Pages: 4

Glaenzel, A.; Adsorption / desorption of CGA 279202 in Borstel soil; Syngenta Crop Protection AG, Basel, Switzerland; Report No.: 00AG05; Document No.: M-049477-01-1; 2000-12-12; Pages: 59

Glaenzel, A.; Adsorption/desorption of CGA 321113 in Borstel soil; Syngenta Crop Protection AG, Basel, Switzerland; Report No.: 00AG06; Document No.: M-051381-01-1; 2000-12-12; Pages: 58

Grade, R.; Growth inhibition test of CGA 279202 tech. to green algae (*Scenedesmus subspicatus*) in a static system; Ciba-Geigy Limited, Basel, Switzerland; Report No.: 943533; Document No.: M-032098-01-1; 1995-06-30; Pages: 23

Grade, R.; Toxicity test of CGA 279202 tech. on sediment-dwelling *Chironomus riparius* (syn. Chironomus thummi) under static conditions; Novartis Crop Protection AG, Basel, Switzerland; Report No.: 983812; Document No.: M-033988-01-1; 1998-12-11; Pages: 65

Heim, L. G.; Velagaleti, R.; Adsorption-desorption of [phenyl (B)-U-14C]-CGA-357276 in soil; ABC Laboratories, Inc., Columbia, MO, USA; Report No.: 210-97; Document No.: M-036507-01-1; 1997-11-22; Pages: 348

Heim, L. G.; Velagaleti, R.; Adsorption-desorption of [phenyl (B)-U-14C]-CGA-357261 in soil; ABC Laboratories, Inc., Columbia, MO, USA; Report No.: 211-97; Document No.: M-036399-01-1; 1997-11-22; Pages: 419

Heim, L. G.; Velagaleti, R.; Adsorption-desorption of [phenyl (B)-U-14C]-CGA-373466 in soil; ABC Laboratories, Inc., Columbia, MO, USA; Report No.: 397-96; Document No.: M-036332-01-1; 1997-11-22; Pages: 430

Herno, V.; Calculation of growth rate endpoints and validity check of trifloxystrobin study on *Lemna gibba* (Ward et al, 1996; M-032662-01-1); Bayer S.A.S., Crop Science Division, Lyon, France; Report No.: M-626098-01-1; Document No.: M-626098-01-1; 2018-06-08; Pages: 11

Herno, V.; Statement - EC10/EC20 calculation for Daphnia reproduction study with trifloxystrobin (Boeri et al, 1996; M-032097-01-1); Bayer AG, Germany; Report No.: M-582255-01-1; Document No.: M-582255-01-1; 2017-02-15; Pages: 27

Herno, V.; Statement - Endpoint re-calculation of *Desmodesmus subspicatus* study with trifloxystrobin (M-032098-01-1, Grade, 1995); Bayer AG, Germany; Report No.: M-582093-01-1; Document No.: M-582093-01-1; 2017-02-16; Pages: 25

Herno, V.; Statement - Endpoint re-calculation of the sediment dwelling organisms study with trifloxystrobin (M-033988-01-1, Grade, 1998); Bayer AG, Germany; Report No.: M-582256-01-1; Document No.: M-582256-01-1; 2017-02-22; Pages: 21

Kitschmann, P.; Aqueous photolysis of [trifluormethyl-phenyl-(U)-14C]-CGA 279202 under laboratory conditions; Novartis Crop Protection AG, Basel, Switzerland; Report No.: 94PK02; Document No.: M-033788-01-1; 1997-11-21; Pages: 168

Kitschmann, P.; Degradation and metabolism of [trifluoromethyl-phenyl-(U)-14C] labeled CGA 279202 in two aquatic systems; Novartis Crop Protection AG, Basel, Switzerland; Report No.: 95PK03; Document No.: M-033933-01-1; 1997-07-15; Pages: 148

Kitschmann, P.; Hydrolysis of (U)-14C-phenyl-glyoxylate-labeled CGA 279202 under laboratory conditions; Ciba-Geigy Limited, Basel, Switzerland; Report No.: 94PK01; Document No.: M-033720-01-1; 1996-12-16; Pages: 240

Mohamed Kamal, B.; Farag Rashed, R.; Mohamed Erasha, A.; Development of sternum and ribs in white New Zealand rabbit (Oryctolagus cuniculus); University of Sadat City, Sadat City, Egypt; Report No.: M-625031-01-1; Document No.: M-625031-01-1; 2016-09-25; Pages: 9

Neumann, C.; Acute toxicity of CGA 279202 WG 50 (A-9360 B) to the cladoceran *Daphnia magna* Straus under static conditions; Novartis Crop Protection AG, Basel, Switzerland; Report No.: 963624; Document No.: M-051484-01-1; 1997-09-15; Pages: 45

Palmer, A. K.; Developmental abnormalities - Rabbits; Benirschke, K.; Garner, F.M.; Jones, T. C.; Report No.: MO-03-002412; Document No.: M-081530-01-1; 1978-12-31; Pages: 14

Perez-Cano, F.; Franch, A.; Castellote, C.; Castell, M.; The suckling rat as a model for immunonutrition studies in early life; University of Barcelona, Barcelona, Spain; Report No.: M-618116-01-1; Document No.: M-618116-01-1; 2012-12-31; Pages: 18

Phaff, R.; Rate and quantum yield of the direct phototransformation of CGA 279202 under laboratory conditions in water; Novartis Crop Protection AG, Basel, Switzerland; Report No.: 96RP03; Document No.: M-033847-02-1; 1998-01-08; Pages: 38

Reinken, G.; Maassen, K.; Kinetic evaluation of degradation and dissipation behaviour of trifloxystrobin and its metabolite CGA 321113 in water / sediment systems according to FOCUS kinetics using the KinGUI 2 tool; Bayer CropScience AG, Monheim, Germany; Report No.: EnSa-13-0736; Document No.: M-468895-01-1; 2013-11-04; Pages: 216

Ryser, M.; Report on surface tension of aqueous solutions - CGA 279202; Novartis Crop Protection AG, Basel, Switzerland; Report No.: PP-97/23T.SUR; Document No.: M-043058-01-1; 1997-08-25; Pages: 8

Schaeffer, A.; Adsorption/desorption of (U)-14C-phenyl-glyoxylate labeled CGA 279202 in various soil types; Ciba-Geigy Limited, Basel, Switzerland; Report No.: 94AS01; Document No.: M-033549-03-1; 1996-07-30; Pages: 79

Schaeffer, A.; Adsorption/desorption of (U)-14C-phenyl-glyoxylate-labeled CGA 321113 in various soil types; Ciba-Geigy Limited, Basel, Switzerland; Report No.: 94AS03; Document No.: M-033569-02-1; 1996-08-14; Pages: 76

Schaeffer, A.; Aqueous photolysis of [glyoxyl-phenyl-(U)-14C]-CGA 279202 under laboratory conditions; Ciba-Geigy Limited, Basel, Switzerland; Report No.: 94AS02; Document No.: M-033754-02-1; 1996-07-30; Pages: 169

Sneikus, J.; Photolysis of trifloxystrobin in natural water; Bayer CropScience AG, Monheim, Germany; Report No.: MEF-247/03; Document No.: M-106330-01-1; 2003-09-30; Pages: 58

Solà, J.; [Trifluoromethylphenyl-UL-14C]Trifloxystrobin: Metabolic stability and profiling in liver microsomes from rats and humans for inter-species comparison; Harlan Laboratories S.A., Barcelona, Spain; Report No.: EnSa-13-0823; Document No.: M-473161-02-1; 2015-03-16; Pages: 69

Stroech, K.; Weuthen, M.; [Benzeneacetic-phenyl-UL-14C]BCS-CU98569 (sodium salt of CGA 381318): Adsorption / desorption on four European soils; Bayer CropScience AG, Monheim, Germany; Report No.: EnSa-12-0384; Document No.: M-447879-01-1; 2013-02-22; Pages: 92

Stroech, K.; Weuthen, M.; [Benzonitrile-ring-UL-14C]NOA 409480: Adsorption / desorption on four European soils; Bayer CropScience AG, Monheim, Germany; Report No.: EnSa-12-0383; Document No.: M-442865-01-1; 2012-11-28; Pages: 94

Stulz, J.; Report on dissociation constant in water - CGA 279202; Novartis Crop Protection Muenchwilen AG, Muenchwilen, Switzerland; Report No.: 46883; Document No.: M-041749-01-1; 1997-04-02; Pages: 6

Stulz, J.; Report on octanol / water partition coefficient - CGA 279202; Novartis Crop Protection Muenchwilen AG, Muenchwilen, Switzerland; Report No.: 46884; Document No.: M-041647-01-1; 1997-02-28; Pages: 10

Stulz, J.; Report on solubility in organic solvents - CGA 279202; Novartis Crop Protection Muenchwilen AG, Muenchwilen, Switzerland; Report No.: 53276; Document No.: M-041631-01-1; 1997-09-03; Pages: 3

Stulz, J.; Report on water solubility - CGA 279202; Novartis Crop Protection Muenchwilen AG, Muenchwilen, Switzerland; Report No.: 46885; Document No.: M-041593-01-1; 1997-02-12; Pages: 8

Stulz, J.; Solubility in organic solvents; Ciba-Geigy Limited, Muenchwilen, Switzerland; Report No.: MO-01-003795; Document No.: M-041643-01-1; 1996-12-11; Pages: 1

Tinnefeld, D.; [Benzeneacetic-phenyl-UL-14C]NOA 413161: Adsorption/desorption on four soils; Bayer CropScience AG, Monheim, Germany; Report No.: MEF-09/479; Document No.: M-361829-01-1; 2010-01-18; Pages: 83

Tinnefeld, D.; [Benzeneacetic-phenyl-UL-14C]NOA 413163: Adsorption/desorption on four soils; Bayer CropScience AG, Monheim, Germany; Report No.: MEF-09/518; Document No.: M-361835-01-1; 2010-01-18; Pages: 83

Ulbrich, R.; Degradation and metabolism of (U)-14-C-phenyl-glyoxylat-labeled CGA 279202 in two aquatic systems; Novartis Crop Protection AG, Basel, Switzerland; Report No.: 95UL02; Document No.: M-033922-01-1; 1997b-11-24; Pages: 132

Ulbrich, R.; Hydrolysis of (trifluormethyl-phenyl-(U)-14C)-labeled CGA 279202 under laboratory conditions; Novartis Crop Protection AG, Basel, Switzerland; Report No.: 94UL04; Document No.: M-033737-01-1; 1997a-12-05; Pages: 307

United States Environmental Protection Agency (USEPA) 2006. *Standard Soil Mobility Classification Guidance.* Memorandum From S. Bradbury to Environmental Fate and Effects Division. January 23, 2004. Environmental Fate and Effects Division. Office of Pesticide Programs.

Ward, T. J.; Magazu, J. P.; Boeri, R. L.; Acute toxicity of CGA 279202 to the crayfish, *Procambarus acutus acutus*; Wilbury Laboratories, Inc., Marblehead, MA, USA; Report No.: 335-98; Document No.: M-052687-01-1; 1998-06-17; Pages: 28

Ward, T. J.; Magazu, J. P.; Boeri, R. L.; Toxicity of CGA 279202 to the duckweed, *Lemna Gibba* G3; Wilbury Laboratories, Inc., Marblehead, MA, USA; Report No.: 671-CG; Document No.: M-032662-01-1; 1996-11-04; Pages: 32

Weinstock, M.; Report on the test for ready biodegradability of CGA 279202 tech. in the carbondioxide evolution test; Ciba-Geigy Limited, Basel, Switzerland; Report No.: 943535; Document No.: M-033914-01-1; 1994-09-19; Pages: 15

Widmer, H.; Vapour pressure of CGA 279202; Ciba-Geigy Limited, Basel, Switzerland; Report No.: 96WI29; Document No.: M-041511-01-1; 1996-11-27; Pages: 22

15 APPENDICES & ANNEXES

Appendix 1: HISTORICAL CONTROL INCIDENCE OF STERNAL FINDINGS IN RUSSIAN (CHBB:HM) RABBITS

Appendix 2: ANALYSIS OF RABBIT FETAL BODY WEIGHTS AND STERNAL FINDINGS

Annex I: Separate document - robust study summaries

Annex II: Separate document - confidential references

APPENDIX 1: HISTORICAL CONTROL INCIDENCE OF STERNAL FINDINGS IN RUSSIAN (CHBB:HM) RABBITS

Historical control data taken from report M-000780-01-1 (Anonymous 1996b)

6 studies performed between 1991 – 1993 with CHBB:HM rabbits (87 litters with 569 fetuses examined) at [redacted].

Table 35: Historical control data – Asymmetrically shaped sternebrae

Parameter		Study						
		T3039597 1991	T4040262 1991	T4040127 1991	T4040749 1992	T1039496 1993	T5044250 1993	**Overall Range**
No. fetuses		95	99	90	80	115	90	569
No. litters		14	15	14	13	15	16	87
Asymmetrically shaped sternebra(e)								
2nd segment	Fetuses affected [N]	-	-	-	1	-	-	0 – 1
	Fetal incidence [%]	-	-	-	1.3	-	-	0.0 – 1.3
	Litters affected [N]	-	-	-	1	-	-	0 – 1
	Litter incidence [%]	-	-	-	7.7	-	-	0.0 – 7.7
1st – 4th segment	Fetuses affected [N]	1	-	-	1	-	-	0 – 1
	Fetal incidence [%]	1.1	-	-	1.3	-	-	0.0 – 1.3
	Litters affected [N]	1	-	-	1	-	-	0 – 1
	Litter incidence [%]	7.1	-	-	7.7	-	-	0.0 – 7.7
2nd – 4th segment	Fetuses affected [N]	2	-	-	-	-	2	0 – 2
	Fetal incidence [%]	2.1	-	-	-	-	2.2	0.0 – 2.2
	Litters affected [N]	2	-	-	-	-	2	0 – 2
	Litter incidence [%]	14.3	-	-	-	-	12.5	0.0 – 14.3
2nd – 5th segment	Fetuses affected [N]	-	-	-	1	-	-	0 – 1
	Fetal incidence [%]	-	-	-	1.3	-	-	0.0 – 1.3
	Litters affected [N]	-	-	-	1	-	-	0 – 1
	Litter incidence [%]	-	-	-	7.1	-	-	0.0 – 7.1
2nd – 5, 6th segment	Fetuses affected [N]	1	-	-	-	-	-	0 – 1
	Fetal incidence [%]	1.1	-	-	-	-	-	0.0 – 1.1
	Litters affected [N]	1	-	-	-	-	-	0 – 1
	Litter incidence [%]	7.1	-	-	-	-	-	0.0 – 7.1
Sum	Fetuses affected [N]	**4**	-	-	**3**	-	**2**	**0 – 4**
	Fetal incidence [%]	**4.2**	-	-	**3.8**	-	**2.2**	**0.0 – 4.2**
	Litters affected [N]	**4**	-	-	**3**	-	**2**	**0 – 4**
	Litter incidence [%]	**28.6**	-	-	**23.1**	-	**12.5**	**0.0 – 28.6**

Historical control data taken from report Anonymous (1996b) M-000780-01-1

6 studies performed between 1991 – 1993 with CHBB:HM rabbits (87 litters with 569 fetuses examined) at [redacted]

Table 36: Historical control data – Fused sternebrae

Parameter		Study						
		T3039597 1991	T4040262 1991	T4040127 1991	T4040749 1992	T1039496 1993	T5044250 1993	**Overall Range**
No. fetuses		95	99	90	80	115	90	569
No. litters		14	15	14	13	15	16	87
Fused sternebra(e)								
3rd with 4th segment	Fetuses affected [N]	1	2	1	2	-	1	0 – 2
	Fetal incidence [%]	1.1	2.0	1.1	2.5	-	1.1	0.0 – 2.5
	Litters affected [N]	1	2	1	2	-	1	0 – 2
	Litter incidence [%]	7.1	13.3	7.1	15.4	-	6.3	0.0 – 15.4
4th with 5th segment	Fetuses affected [N]	6	2	-	1	1	4	0 – 6
	Fetal incidence [%]	6.3	2.0	-	1.3	0.9	4.4	0.0 – 6.3
	Litters affected [N]	4	1	-	1	1	3	0 – 4
	Litter incidence [%]	28.6	6.7	-	7.7	6.7	18.8	0.0 – 28.6
2nd – 4th segment	Fetuses affected [N]	-	-	1	-	-	1	0 – 1
	Fetal incidence [%]	-	-	1.1	-	-	1.1	0.0 – 1.1
	Litters affected [N]	-	-	1	-	-	1	0 – 1
	Litter incidence [%]	-	-	7.1	-	-	6.3	0.0 – 7.1
2nd – 5th segment	Fetuses affected [N]	2	-	-	-	-	1	0 – 2
	Fetal incidence [%]	2.1	-	-	-	-	1.1	0.0 – 2.1
	Litters affected [N]	2	-	-	-	-	1	0 – 2
	Litter incidence [%]	14.3	-	-	-	-	6.3	0.0 – 14.3
3rd – 5th segment	Fetuses affected [N]	-	-	1	3	-	1	0 – 3
	Fetal incidence [%]	-	-	1.1	3.8	-	1.1	0.0 – 3.8
	Litters affected [N]	-	-	1	3	-	1	0 – 3
	Litter incidence [%]	-	-	7.1	23.1	-	6.3	0.0 – 23.1
Sum	Fetuses affected [N]	**9**	**4**	**3**	**6**	**1**	**8**	**1 – 9**
	Fetal incidence [%]	**9.5**	**4.0**	**3.3**	**7.5**	**0.9**	**8.9**	**0.9 – 9.5**
	Litters affected [N]	**7**	**3**	**3**	**6**	**1**	**7**	**1 – 7**
	Litter incidence [%]	**50.0**	**20.0**	**21.4**	**46.2**	**6.7**	**43.8**	**6.7 – 50.0**

Additional information on historical control data ((CHBB:HM) Rabbits) taken from report M-039377-03-1 (Anonymous 1996b) (study number 943043) conducted with trifloxystrobin at [redacted]

1. Purpose of the Revised Supplement

The purpose of this supplement is to present historical control data (EC 91/414) of fetal external, visceral and skeletal findings supporting a rabbit oral teratogenicity study (test number 943043) conducted with CGA 279202 technical.

This revised supplement is intended to fully replace the supplement issued October 31, 1997. It was found that incorrect data for skeletal findings were inadvertently included in the original supplement, i.e., data for rats were included rather than data for rabbits. The correct data are presented in this revised supplement. Also, this supplement includes information which more fully describes the characteristics of the studies included, as specified by Directive EC 91/414.

2. Study Characteristics

The following extended list of In-house studies included in this supplement were identified in the testing facility database. The route of administration was by gavage. In-life phase of these studies was within 3 years of the reference study. There were 24 control groups of 20 studies.

2.1. Study Time Frame

Test Number	Dosing Start
890007	09.08.1989
900001	02.01.1990
910021	18.11.1991
940021	16.01.1995
891329	04.06.1990
891418	30.04.1990
891485	16.09.1991
911127	01.07.1991
922822	10.08.1992
922847	11.01.1993
923140	16.08.1993
923154	15.03.1993
923167	29.03.1993
926089	15.06.1992
931152	08.11.1993
935133	14.01.1994
941055	22.08.1994
942119	07.08.1995
943043	31.05.1994
951033	11.09.1995

Additional information on historical control data ((CHBB:HM) Rabbits) taken from report M-039377-03-1 (Anonymous 1996b)(study number 943043) conducted with trifloxystrobin at [redacted]

2.2. Experimental Animals

The experimental animals in all 20 studies had the following characteristics:

Species Rabbit

Strain RUSSIAN Chbb: HM

Approximate age at study begin (days): 90 to 120

Approximate age at necropsy (days): 120 to 150

Acclimation and Husbandry: After arrival in the facility, females were identified by an ear tag, quarantined and acclimated to the facility environment for at least seven days before being placed on study. During quarantine, animals were checked for general health; only healthy animals were placed on study.

All these studies were carried out under optimal hygienic conditions. The animals were housed individually in Heinkel cages with steel slatted floors.

Feed: Pelleted, certified standard feed [redacted] was provided ad libitum. Feed batches are analysed for composition and contaminant levels.

Water: ad libitum via metal spouts. The water quality is routinely checked to standard specifications.

Treatment Period: Animals were administered 4 ml/kg body weight of the vehicle on days 7 to 19 of presumed gestation.

Additional information on historical control data ((CHBB:HM) Rabbits) taken from report M-039377-03-1 (Anonymous 1996b) (study number 943043) conducted with trifloxystrobin at [redacted]

SPECIES RABBIT — HISTORICAL CONTROL DATA

STRAIN RUSSIAN — FETAL SKELETAL ANOMALIES

	FETAL INCIDENCE (%) OVERALL N	MEAN	BY GROUP MIN	MAX	LITTER INCIDENCE (%) OVERALL N	MEAN	BY GROUP MIN	MAX
NUMBER EVALUATED	2562				455			
Live	2562							
Dead	0							
STERNEBRA-1: A FUSED STERNEBRA-1 AND STERNEBRA-2	8	0.3	0.0	2.5	8	1.8	0.0	10.5
STERNEBRA-1: A ASYMMETRICALLY SHAPED STERNEBRA-1	10	0.4	0.0	2.3	10	2.2	0.0	13.3
STERNEBRA-1: A FRAGMENTED STERNEBRA-1	13	0.5	0.0	5.2	9	2.0	0.0	15.8
STERNEBRA-1: A FUSED STERNEBRAE	1	0.0	0.0	1.3	1	0.2	0.0	6.3
STERNEBRA-1: A BIPARTITE STERNEBRA-1	1	0.0	0.0	1.0	1	0.2	0.0	5.9
STERNEBRA-2: A FUSED STERNEBRA-2 AND STERNEBRA-3	25	1.0	0.0	5.7	23	5.1	0.0	20.0
STERNEBRA-2: A ASYMMETRICALLY SHAPED STERNEBRA-2	16	0.6	0.0	4.1	15	3.3	0.0	13.3
STERNEBRA-3: A FUSED STERNEBRA-3 AND STERNEBRA-4	68	2.7	0.0	9.2	57	12.5	0.0	33.3
STERNEBRA-3: A ASYMMETRICALLY SHAPED STERNEBRA-3	14	0.5	0.0	2.7	13	2.9	0.0	10.5
STERNEBRA-4: A FUSED STERNEBRA-4 AND STERNEBRA-5	68	2.7	0.0	8.0	57	12.5	0.0	29.4
STERNEBRA-4: A ASYMMETRICALLY SHAPED STERNEBRA-4	23	0.9	0.0	3.2	22	4.8	0.0	17.6
STERNEBRA-5: A ASYMMETRICALLY SHAPED STERNEBRA-5	18	0.7	0.0	2.7	18	4.0	0.0	17.6
STERNEBRA-5: A FRAGMENTED STERNEBRA-5	1	0.0	0.0	1.3	1	0.2	0.0	7.1
STERNEBRA-5: A BIPARTITE STERNEBRA-5	1	0.0	0.0	1.8	1	0.2	0.0	6.7
STERNEBRA-6: A BIFURCATED STERNEBRA-6	1	0.0	0.0	0.9	1	0.2	0.0	5.0
STERNEBRA-6: A CLEFT STERNUM	1	0.0	0.0	0.9	1	0.2	0.0	5.0
STERNEBRA-6: A ASYMMETRICALLY SHAPED STERNEBRA-6	7	0.3	0.0	2.3	7	1.5	0.0	13.3
CRANIAL BONES: A FUSED FRONTAL AND PARIETAL BONES	1	0.0	0.0	0.9	1	0.2	0.0	5.3
CRANIAL BONES: A FRAGMENTED HYOID BONE	2	0.1	0.0	1.0	2	0.4	0.0	5.9
SHOULDER GIRDLE: A IRREGULAR OSSIFICATION SCAPULA	19	0.7	0.0	7.3	13	2.9	0.0	13.3

	FETAL INCIDENCE (%)				LITTER INCIDENCE (%)			
	OVERALL		BY GROUP		OVERALL		BY GROUP	
	N	MEAN	MIN	MAX	N	MEAN	MIN	MAX
NUMBER EVALUATED	262				455			
Live	262							
Dead	0							
PELVIC GIRDLE: A REDUCED PUBIS	5	0.2	0.0	2.8	5	1.1	0.0	15.8
CERV.VERT.CENTER: A DISPLACED CERVICAL VERT.CENTERS	2	0.1	0.0	1.0	2	0.4	0.0	6.3
CERV.VERT.CENTER: A FUSED CERVICAL VERT.CENTERS	1	0.0	0.0	1.0	1	0.2	0.0	6.3
THOR.VERT.CENTER: A HEMICENTRIC THORACIC VERT.CENTERS	1	0.0	0.0	0.3	1	0.2	0.0	1.6
THOR.VERT.CENTER: A ASYMMETRICALLY SHAPED THORACIC VERT.CENTER	1	0.0	0.0	0.3	1	0.2	0.0	1.6
THOR.VERT.CENTER: A DISPLACED THORACIC VERT.CENTERS	1	0.0	0.0	0.9	1	0.2	0.0	5.3
THOR.VERT.CENTER: A FUSED THE 12. THORACIC AND 1. LUMBAR VERTE	1	0.0	0.0	1.3	1	0.2	0.0	7.1
THOR.VERT.CENTER: A ADDITIONAL THORACIC VERT.CENTERS	1	0.0	0.0	1.2	1	0.2	0.0	5.6
THOR.VERT.ARCH: A MISSING THORACIC VERT.ARCHES	1	0.0	0.0	0.3	1	0.2	0.0	1.6
LUMB.VERT.ARCH: A FUSED LUMBAR VERT.ARCHES	1	0.0	0.0	1.3	1	0.2	0.0	5.3
CAUD.VERT.CENTER: A FUSED CAUDAL VERT.CENTERS	22	0.9	0.0	5.0	21	4.6	0.0	22.2
CAUD.VERT.CENTER: A ASYMMETRICALLY SHAPED CAUDAL VERT.CENTERS	11	0.4	0.0	1.8	11	2.4	0.0	11.1
CAUD.VERT.CENTER: A DISPLACED CAUDAL VERT.CENTERS	41	1.6	0.0	5.4	40	8.8	0.0	33.3
CAUD.VERT.CENTER: A BIPARTITE CAUDAL VERT.CENTERS	1	0.0	0.0	1.0	1	0.2	0.0	6.3
CAUD.VERT.CENTER: A FRAGMENTED CAUDAL VERT.CENTRES	1	0.0	0.0	0.9	1	0.2	0.0	5.9
RIBS: A KINKED RIBS	1	0.0	0.0	0.9	1	0.2	0.0	5.0
RIBS: A SHORTENED RIBS	6	0.2	0.0	1.8	6	1.3	0.0	10.0
RIBS: A IRREGULAR OSSIFICATION RIBS	1	0.0	0.0	0.9	1	0.2	0.0	5.0
RIBS: A MISSHAPEN RIB(S)	1	0.0	0.0	0.9	1	0.2	0.0	5.0
RIBS: A MISSING RIBS	2	0.1	0.0	0.9	2	0.4	0.0	5.0
TOTAL FETAL SKELETAL ANOMALIES	239	9.3			173	38.0		

APPENDIX 2: ANALYSIS OF RABBIT FETAL BODY WEIGHTS AND STERNAL FINDINGS

Please refer to section 10.10.5.2.

An evaluation of the body weight and body weight changes of individual dams producing litters with sternal findings and evaluation of individual pup weights is presented below.

Five out of 8 and 4 out of 6 dams producing litters with sternal findings in the 250 and 500 mg/kg bw/day groups, respectively, exhibited body weight losses greater than the group mean from the start of dosing to the day after the last dose (days 7-20). There was no obvious correlation with starting weight (Day 0) or carcass weight and this was also the case for the control and lower dose groups. Examination of the affected fetuses in the 250 and 500 mg/kg bw/day groups showed that the majority of the fetuses had weights lower than the group mean and they were often amongst the smallest in their respective litters. Although there wasn't a consistent pattern of smaller fetuses (within each litter) among affected fetuses in the control and lower dose groups, the majority of affected fetuses had lower weights than the group mean. Fetuses with fusion of sternebrae 1-4 or 1-5 tended to be the smallest in their respective litters.

This evaluation indicates that the background variation in dam body weights and body weight changes is not correlated with the spontaneous occurrence of fetuses with sternal findings, as exemplified by the pattern observed in the control and lower dose groups (with no treatment related effect on maternal weights). The pattern of sternal findings was identical between the affected control animals and those treated with trifloxystrobin.

Therefore, the increased incidence of sternal variations in the 250 and 500 mg/kg bw/day groups is probably related to maternal toxicity (body weight losses) and subsequent effects on fetal weight (delayed development). Whilst there wasn't an overall treatment related effect on mean fetal weight in these two groups it is clear that there was a relatively high incidence of lower weight fetuses amongst those exhibiting sternal findings, and a significant proportion of affected dams exhibited higher than average body weight losses. Thus, it is considered that maternal toxicity exacerbated the spontaneous occurrence of fetal sternal findings and they are therefore considered to be secondary effects of maternal toxicity and not a direct effect of trifloxystrobin on the fetus.

Skeletal variations occurred in about two thirds of fetuses from almost all litters in all dose groups. They consisted mainly of poor or absent ossification of sternebra-l, -5, -6, cranial findings (sutural bones, slot or hole in parietal bone), absent ossification of metacarpal-l, tail bone variations (poor or absent ossification of or additional caudal vertebral centres), additional ribs, and poor ossification of the medial phalanx of anterior digit-5. Poor ossification of the caudal vertebral centres showed statistically significant higher values for the low-mid dose group when compared to controls. However, since there was no dose-relationship and since these values were within the historical control ranges they were considered not to be treatment related; historical database of 2562 fetuses and 455 litters (fetal % incidence/litter % incidence ranges: 3.2-27.7%/17.6-76.5%).

Table 37: Body weights (g) of selected dams and of fetuses showing sternal findings

Dam no.	Bw Day 0	Bw change Day 7-20	Carcass weight	Litter size	Fetus no.	Fetal weight	Comment[a]
Group 1 (control)							
7	2658	70	**2536**	9	8	**29.4**	Smallest (Fusion 1-5)
9	**2536**	**52**	**2398**	7	1	**33.0**	Smallest
10	2773	**-100**	**2407**	7	1	**38.7**	Average
11	2853	145	2700	5	5	47.0	Heaviest
17	**2531**	96	**2373**	7	2	**39.7**	Average
G mean	*2715*	*64*	*2579*	*6.1*		*40.0*	
Group 2 (10 mg/kg bw/day)							
25	2845	**-25**	2632	7	3	41.4	Heaviest
					6	**30.1**	Smallest (Fusion 1-4 + asym)
32	2747	**22**	**2484**	8	7	**33.4**	2nd heaviest
36	**2705**	**49**	2555	5	1	**30.9**	Smallest
G mean	*2707*	*59*	*2544*	*7.2*		*37.3*	
Group 3 (50 mg/kg bw/day)							
39	2829	45	2639	7	7	**28.9**	Smallest
48	2775	**22**	2647	4	2	45.9	Heaviest
51	2756	84	2725	9	9	**33.0**	Average
55	**2634**	40	**2512**	7	1	**15.8**	Smallest (Fusion 1-5 + asym)
G mean	*2737*	*34*	*2611*	*5.6*		*40.2*	
Group 4 (250 mg/kg bw/day)							
62	2951	**-126**	2864	2	3	42.1	Smallest
63	2863	27	2652	8	1	40.4	2nd heaviest
					7	**31.9**	Smallest (Fusion 1-5 + asym)
					8	41.0	Heaviest
65	**2534**	1	**2319**	6	4	**30.5**	Smallest
					6	**31.6**	2nd smallest
66	2877	**-298**	2683	6	7	**26.1**	Smallest
67	2803	**-227**	**2347**	7	7	**36.3**	2nd heaviest
71	2765	**-203**	2586	11	1	**31.8**	Average
					7	**32.2**	Average
72	**2380**	**-102**	**2322**	3	4	**35.1**	Smallest
74	**2702**	-29	2618	7	4	**37.2**	2nd smallest
G mean	*2703*	*-83*	*2533*	*5.4*		*38.2*	
Group 5 (500 mg/kg bw/day)							
82	3043	**-240**	2897	3	3	41.8	Smallest
84	2880	**-412**	**2284**	8	1	**27.6**	Smallest
					2	**32.0**	Average
85	**2538**	**-200**	**2386**	6	5	43.7	Average
86	2769	-39	2592	5	3	39.6	Heaviest
					7	**37.8**	Smallest
87	2827	-91	2592	8	5	**33.2**	3rd smallest
93	**2584**	**-164**	**2349**	11	1	**31.4**	5th small. (Fusion 1-5 + asym)
					2	**30.4**	4th smallest
					5	**26.9**	3rd smallest
					6	**24.1**	2nd smallest
					8	**15.4**	Smallest
G mean	*2724*	*-152*	*2525*	*5.1*		*39.3*	

[a] Comment on relative weight of the fetus in the litter (fusion of several sternebrae ± asymmetrically shaped sternebrae)
Body weights in **bold** are lower than the group mean (G mean)