Committee for Risk Assessment

RAC

Annex 1

Background document

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

pyraclostrobin (ISO); methyl *N*-(2-{[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl]oxymethyl}phenyl) *N*-methoxy carbamate

EC Number: -
CAS Number: 175013-18-0

CLH-O-0000007219-70-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to 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

1 December 2022

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

Pyraclostrobin (ISO); methyl N-(2-{[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl]oxymethyl}phenyl) *N*-methoxy carbamate

EC Number: -

CAS Number: 175013-18-0

Index Number: -

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Version number: 2.0 **Date: September 2021**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PYRACLOSTROBIN (ISO); METHYL N-(2-{[1-(4-CHLOROPHENYL)-1H-PYRAZOL-3-YL]OXYMETHYL}PHENYL) N-METHOXY CARBAMATE

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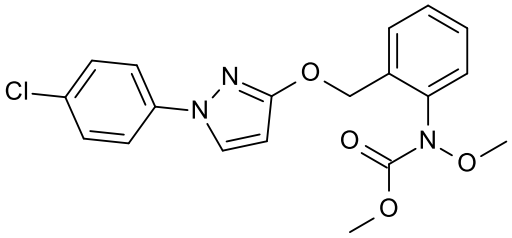
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PYRACLOSTROBIN (ISO); METHYL N-(2-{[1-(4-CHLOROPHENYL)-1H-PYRAZOL-3-YL]OXYMETHYL}PHENYL) N-METHOXY CARBAMATE

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)	Methyl N-(2-{[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl]oxymethyl}phenyl) *N*-methoxy carbamate
Other names (usual name, trade name, abbreviation)	Carbamic acid, [2-[[[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy]methyl]phenyl]methoxy-, methyl ester
ISO common name (if available and appropriate)	Pyraclostrobin (ISO)
EC number (if available and appropriate)	-
EC name (if available and appropriate)	-
CAS number (if available)	175013-18-0
Other identity code (if available)	CIPAC: 657
Molecular formula	$C_{19}H_{18}ClN_3O_4$
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	387.82 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	
Description of the manufacturing process and identity of the source (for UVCB substances only)	
Degree of purity (%) (if relevant for the entry in Annex VI)	Minimum purity: 97.5

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)
Pyraclostrobin CAS: 175013-18-0		-	Acute Tox. 3, H331 STOT SE 3 Skin Irrit. 2 Aquatic Acute 1, M = 100 Aquatic Chronic 1, M = 10

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Toluene EC: 203-625-9 CAS: 108-88-3	1 %	Flam. Liq. 2 Skin Irrit. 2 Asp. Tox. 1 STOT SE 3 STOT RE 2* Repr. 2		No
Dimethyl sulphate EC: 201-058-1 CAS: 77-78-1	0.0001 %	Acute Tox. 3; H301 Skin Corr. 1B Skin Sens. 1 Acute Tox. 2; H330 Muta. 2; C ≥ 0.01 % Carc. 1B; C ≥ 0.01 % STOT SE 3, C ≥ 5 %		No

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
None					

Table 5: Test substances (non-confidential information) (this table is optional)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6: Proposed harmonised classification and labelling according to the CLP criteria

	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	613-272-00-6	pyraclostrobin (ISO); methyl N-(2-{[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl]oxymethyl}phenyl) *N*-methoxy carbamate	-	-	Acute Tox. 3 * Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1	H331 H315 H400 H410	GHS06 GHS09 Dgr	H331 H315 H410		M=100	
Dossier submitter's proposal	613-272-00-6	pyraclostrobin (ISO); methyl N-(2-{[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl]oxymethyl}phenyl) *N*-methoxy carbamate	-	175013-18-0	Repr. 2 Acute Tox. 3 Acute Tox. 4 STOT SE 3 STOT RE 2 Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1	H361d H331 H302 H335 H373 (liver, gastrointestinal tract) H315 H400 H410	GHS08 GHS06 GHS09 Dgr	H361d H331 H302 H335 H373 (liver, gastrointestinal tract) H315 H410	-	oral: ATE = 450 mg/kg bw inhalation: ATE = 0.58 mg/L (dusts or mists) M = 100 M = 100	
Resulting Annex VI entry if agreed by RAC and COM	613-272-00-6	pyraclostrobin (ISO); methyl N-(2-{[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl]oxymethyl}phenyl) *N*-methoxy carbamate	-	175013-18-0	Repr. 2 Acute Tox. 3 Acute Tox. 4 STOT SE 3 STOT RE 2 Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1	H361d H331 H302 H335 H373 (liver, gastrointestinal tract) H315 H400 H410	GHS08 GHS06 GHS09 Dgr	H361d H331 H302 H335 H373 (liver, gastrointestinal tract) H315 H410	-	oral: ATE = 450 mg/kg bw inhalation: ATE = 0.58 mg/L (dusts or mists) M = 100 M = 100	

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

Hazard class	**Reason for no classification**	**Within the scope of standard consultation**
Explosives	Data lacking	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Hazard class not applicable	No
Flammable solids	Data conclusive but not sufficient for classification	Yes
Self-reactive substances	Data lacking.	Yes
Pyrophoric liquids	Hazard class not applicable	No
Pyrophoric solids	Data conclusive but not sufficient for classification	Yes
Self-heating substances	Data lacking.	Yes
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	Yes
Oxidising liquids	Hazard class not applicable	No
Oxidising solids	Data lacking	Yes
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Hazard class not applicable.	No
Desensitised explosives	Data lacking	Yes
Acute toxicity via oral route	Harmonised classification proposed	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Harmonised classification proposed	Yes
Skin corrosion/irritation	Harmonised classification proposed	Yes
Serious eye damage/eye irritation	Data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	Data lacking	Yes
Skin sensitisation	Data conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	Data conclusive but not sufficient for classification	Yes
Reproductive toxicity	Harmonised classification proposed	Yes
Specific target organ toxicity-single exposure	Harmonised classification proposed	Yes

Hazard class	Reason for no classification	Within the scope of standard consultation
Specific target organ toxicity-repeated exposure	Harmonised classification proposed	Yes
Aspiration hazard	Hazard class not applicable	No
Hazardous to the aquatic environment	Harmonised classification proposed	Yes
Hazardous to the ozone layer	Data lacking	Yes

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

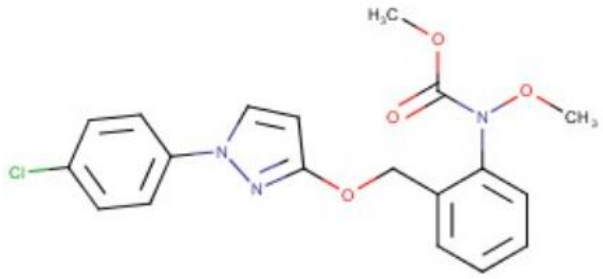
The substance ~~was not subject to harmonised classification and labelling before~~ has an existing entry in Annex VI to the CLP Regulation.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The substance is an active substance in the meaning of Regulation EC 1107/2009 or Regulation (EU) No 528/2012 which shall normally be subject to harmonised classification and labelling, and justification is not required. (Article 36 CLP Regulation).

RAC general comment

Pyraclostrobin (ISO) is a fungicidal active substance used in plant protection products.



Structure of pyraclostrobin

Pyraclostrobin is a white or light beige solid at room temperature with a melting point range of 63.7 °C – 65.2 °C, a water solubility of 1.9 ± 0.17 mg/L (at 20 °C in deionized water, pH of 5.8) and a log K_{ow} of 3.99.

Toxicokinetics

Three *in vivo* studies via oral administration (an OECD test guideline TG 417 study each in Wistar rats and NMRI mouse and a metabolism study in Wistar rats according to the method EEC 87/302) and a comparative *in vitro* metabolism study are reported in the CLH report for pyraclostrobin.

After a single dose of 5 or 50 mg ^{14}C-radiolabeled pyraclostrobin/kg bw to Wistar rats, oral absorption was found to be rapid but incomplete. At 120 h post-dosing, only 15 % of the applied radioactivity was excreted via the urine, whereas elimination via the faeces accounted for 80-90 % of the dose. With the biliary excretion of approx. 35% determined at 48 h post-dosing, an oral bioavailability of 50% is assumed for both sexes. There was no evidence of accumulation of pyraclostrobin in this study and the highest amount of radioactivity was found in the gastro-intestinal tract followed by the liver. All other tissues had values comparable to or less than the plasma concentrations (TOX2000-705: 1998).

After a single oral dose of 300 mg radiolabelled pyraclostrobin/kg bw to NMRI mouse, radioactivity was detectable after 2 h in the systemic circulation, in the bone marrow and in the liver (ASB2017-5506: 2016).

The systemically available portion of pyraclostrobin was rapidly and extensively metabolised in Wistar rats. N-desmethoxylation was the quantitatively most important pathway. No major differences were observed with regard to sex and dose level (TOX2000-708: 1999).

In a comparative *in vitro* metabolism study in hepatocytes from humans, rabbits, rats and dogs, many similarities but also remarkable differences were noted for pyraclostrobin. At the highest concentration of 10 µM, the cytotoxicity in human cells was much greater compared to that in rats and rabbits. However, there was no difference noted at the lower concentration of 3 µM. Metabolism of pyraclostrobin appeared faster in cells and microsome preparations obtained from rabbits and rats as compared to those from humans and it was slowest in dog microsomes. Qualitatively, the rabbit metabolite profile was most similar to that of humans. A few metabolites that were abundant in human and rabbit cells were not identified in rat samples (ASB2015-8295: 2014).

5 IDENTIFIED USES

Pyraclostrobin is used as a fungicidal agent in plant protection products.

6 DATA SOURCES

Renewal Assessment Report – Pyraclostrobin – Volume 3 – B.8 Environmental fate and behaviour, Rev. 1, 2020.

Renewal Assessment Report – Pyraclostrobin – Volume 3 – B.9 Ecotoxicology data, Rev. 1, 2020

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	white or light beige solid (at room temperature)	CHE2000-471: Tuerk, 1996	Visual assessment (Purity: 99.8)
	Pyraclostrobin technical material (amorphous) was determined to be a solid, amber, glass like material.	2679541: Kroehl, 2013	Visual assessment (Purity: 96.8)
	Pyraclostrobin technical material (crystalline) was determined to be a solid, light yellow, odourless, fine crystalline powder.	2679542: Kroehl, 2010	Visual assessment (Purity: 99.4)
Melting/freezing point	Melting point (range): 63.7 °C – 65.2 °C	CHE2000-471: Tuerk, 1996	EC A 1 (DSC) (Purity: 99.8)
Boiling point			Not applicable for a solid
Relative density			
Vapour pressure	2.6 x 10^{-8} Pa at 20 °C 6.4 x 10^{-8} Pa at 25 °C	CHE2000-470: Kaestel, 1998	EC A 4 (Balance method) (Purity: 99.8)
Surface tension	71.8 mN/m at 0.5 % (w/w) (20 °C) 71.5 mN/m at 2.0 % (w/w) (20 °C)	CHE2000-470: Kaestel, 1998	EC A 5 (Purity: 98.5)
Water solubility	1.9 ± 0.17 mg/L at 20 °C in deionized water at a pH of 5.8. There is no dissociation in water therefore pH dependence on solubility is not applicable.	CHE2000-467: Tuerk, 1996	EC A 6 (column elution method) (Purity: 99.8)
Partition coefficient n-octanol/water	The mean log P_{OW} was 3.99 and the corresponding P_{OW} was 9772. Effect of pH was not investigated since there is no dissociation in water.	CHE2000-465: Tuerk, 1996	OECD 117 (HPLC) (Purity: 99.8)
	The following studies/data for metabolites included in the residue definition for		

Property	Value	Reference	Comment (e.g. measured or estimated)
	risk assessment have been provided. Log $P_{o/w}$ data of the metabolites BF 500-4, BF 500-6, BF 500-7, BF 500-11, BF 500-13, BF 500-14 are missing.		
	Metabolite M500F004 (synonym: 500M04, BF 500-5, Reg.No. 298327): log $P_{o/w}$ = 1.8 (20 °C; pH 6) log $P_{o/w}$ = 1.8 (20 °C; pH 4) log $P_{o/w}$ = 1.0 (20 °C; pH 7) log $P_{o/w}$ = 0.8 (20 °C; pH 9)	3820386: Daum, 2018	(Purity: 99.6) OECD 117 (HPLC)
	Metabolite M500F007 (synonym: 500M07, BF 500-3, Reg.No. 340266): log $P_{o/w}$ = 3.7 (20 °C; pH 6) log $P_{o/w}$ = 3.7 (20 °C; pH 4) log $P_{o/w}$ = 3.7 (20 °C; pH 7) log $P_{o/w}$ = 3.8 (20 °C; pH 9)	3820388: Daum, 2018	OECD 117 (HPLC) (Purity: 99.9)
	Metabolite M500F085 (synonym: 500M85, BF 500-8, Reg.No. 399530): log $P_{o/w}$ = 2.0 (20 °C; pH 6) log $P_{o/w}$ = 2.0 (20 °C; pH 4) log $P_{o/w}$ = 0.5 (20 °C; pH 7) log $P_{o/w}$ = 0.5 (20 °C; pH 9)	3820390: Daum, 2018	OECD 117 (HPLC) (Purity: 99.3)
Flash point	Not required. Melting point of the technical active substance (TAS) is >40 °C. The flash point of TAS was found to be 132 °C.	CHE2000-470: Kaestel, 1998	EC A 9 (Pensky-Martens method) (Purity: 98.5)

Property	Value	Reference	Comment (e.g. measured or estimated)
Flammability	TAS is not considered highly flammable, it did not burn under test conditions.	CHE2000-464: Loeffler, 1998	EC A 10 (Purity: 98.5)
	Pyraclostrobin technical (solidified melt) was not determined to be highly flammable.	2679557: Achhammer, 2013	EC A 10 (Purity: 99.0)
	Pyraclostrobin technical (crystalline) was not determined to be highly flammable.	2679549: Loehr, 2011 2679554: Loehr, 2011	EC A 10 (Purity: 99.9)
	EC A 10 is in the preliminary test equivalent to UN N.1. Therefore, the test substance is not considered highly flammable.	3820380: Achhammer, 2019	Statement
Explosive properties	Pyraclostrobin technical (solidified melt) is not explosive.	2679557: Achhammer, 2013	EC A 14 (Purity: 99.0)
	Pyraclostrobin technical (crystalline) is not explosive.	2679549: Loehr, 2011 2679554: Loehr, 2011	EC A 14 (Purity: 99.9)
	No self-ignition up to 400 °C.	2679557: Achhammer, 2013	EC A 16 (Purity: 99.0)
Self-ignition temperature	No self-ignition up to 400 °C.	2679549: Loehr, 2011 2679554: Loehr, 2011	EC A 16 (Purity: 99.9)
	The study of oxidizing properties indicates that the active substance is not considered to be an oxidizing agent.	CHE2000-464: Loeffler, 1998	EC A 17 (Purity: 98.5)
Oxidising properties	Pyraclostrobin technical (solidified melt) is not an oxidising substance.	2679557: Achhammer, 2013	EC A 17 (Purity: 99.0)

Property	Value	Reference	Comment (e.g. measured or estimated)
	Pyraclostrobin technical (crystalline) is not an oxidising substance.	2679549: Loehr, 2011 2679554: Loehr, 2011	EC A 17 (Purity: 99.9)
Granulometry			No evidence of instability in organic solvents. Not required.
Stability in organic solvents and identity of relevant degradation products	5.307 x 10^{-9} kPa m^3/ mol at 20 °C	LUF2000-248: Ohnsorge, 2000	Calculation (Purity: 99.8)
Dissociation constant			Not applicable for a solid
Viscosity			

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 9: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EC A 14	Pyraclostrobin technical (solidified melt) is not explosive.	None	2679557: Achhammer, 2013
EC A 14	Pyraclostrobin technical (crystalline) is not explosive.	None	2679549: Loehr, 2011 2679554: Loehr, 2011

8.1.1 Short summary and overall relevance of the information provided on explosive properties

Pyraclostrobin was tested for explosive properties using the EC Method A.14 and was found not to be explosive.The DCS result shows a energy release over 1200 J/g. The following test according to the EEC A.14 were all negative.

8.1.2 Comparison with the CLP criteria

Method EC A.14 is not sufficient on its own to conclude on explosive properties.

Pyraclostrobin does carry functional groups listed in tables A6.1 in Annex 6 (Screening Procedures) of the Manual of Tests and Criteria associated with explosive properties and the calculated oxygen balance is not less than -200. Resulting from this it cannot be excluded that the substance might have explosive properties. According to the CLP Regulation, explosive properties are tested using UN test series 2 to 8 (see Annex I, 2.1.2.3.). Corresponding UN test results are not available.

Since no further information/test result is available, data are not sufficient for the assessment of the explosive properties.

8.1.3 Conclusion on classification and labelling for explosive properties

Data not sufficient for conclusion/classification

8.2 Flammable gases (including chemically unstable gases)

Hazard class not applicable.

8.3 Oxidising gases

Hazard class not applicable.

8.4 Gases under pressure

Hazard class not applicable.

8.5 Flammable liquids

Hazard class not applicable.

8.6 Flammable solids

Table 10: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EC A 10	TAS is not considered highly flammable, it did not burn under test conditions.	None	CHE2000-464: Loeffler, 1998
EC A 10	Pyraclostrobin technical (solidified melt) was not determined to be highly flammable.	None	2679557: Achhammer, 2013
EC A 10	Pyraclostrobin technical (crystalline) was not determined to be highly flammable.	None	2679549: Loehr, 2011 2679554: Loehr, 2011

8.6.1 Short summary and overall relevance of the provided information on flammable solids

Pyraclostrobin is not considered to be highly flammable. It did not burn under test conditions.

8.6.2 Comparison with the CLP criteria

The data of the EC A 10 tests indicate that a classification as highly flammable does not apply. Since the available data from an A.10 test method indicate that a classification as a flammable solid does not apply (result: not highly flammable), no more testing is necessary (see ECHA Guidance, Chapter R.7a: Endpoint specific guidance, R.7.1.10.3).

8.6.3 Conclusion on classification and labelling for flammable solids

Pyraclostrobin is not highly flammable.

8.7 Self-reactive substances

8.7.1 Short summary and overall relevance of the provided information on self-reactive substances

Pyraclostrobin carries functional groups listed in table A6.1 in Annex 6 (Screening Procedures) of the Manual of Tests and Criteria, which are associated with explosive properties and it does carry functional groups listed in table A6.3 in Annex 6 of the UN RTDG indicating self-reactive properties.

8.7.2 Comparison with the CLP criteria

According to CLP Regulation, self-reactive properties are tested using UN test series A to H; the hazard class can be assessed also based on the criteria in CLP Annex I, 2.8.4.2. Since no corresponding UN test results are available and the substance does contain the above mentioned groups, it cannot be excluded that the substance has self-reactive properties. Data are not sufficient for conclusion/classification.

8.7.3 Conclusion on classification and labelling for self-reactive substances

Data not sufficient for conclusion/classification.

8.8 Pyrophoric liquids

Hazard class not applicable.

8.9 Pyrophoric solids

8.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

No test results available. The experience in handling shows that Pyraclostrobin does not ignite spontaneously on coming into contact with air at normal temperatures.

8.9.2 Comparison with the CLP criteria

According to CLP, pyrophoric solids are tested using UN N.2 method (results from test method EU A.13 are acceptable as the two methods are considered equivalent). Corresponding test results are not available. Alternatively, based on experience in handling, the classification procedure for pyrophoric solids does not need to be applied.

8.9.3 Conclusion on classification and labelling for flammable solids

Pyraclostrobin has no pyrophoric properties

8.10 Self-heating substances

Table 11: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EC A 16	No self-ignition up to 400 °C.		2679557: Achhammer, 2013

Method	Results	Remarks	Reference
EC A 16	No self-ignition up to 400 °C.		2679549: Loehr, 2011 2679554: Loehr, 2011

8.10.1 Short summary and overall relevance of the provided information on self-heating substances

The data from a test EC A.16: Up to the melting point no self-heating has been detected.

8.10.2 Comparison with the CLP criteria

The data from a test EC A.16 indicate that a classification as a self-heating substance does not apply. According to CLP Regulation, self-reactive properties are tested using UN test N.4. No corresponding UN test results are available. Results from method EC A.16 are not conclusive to assess this hazard class.

8.10.3 Conclusion on classification and labelling for self-heating substances

Data not sufficient for conclusion/classification.

8.11 Substances which in contact with water emit flammable gases

8.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

The chemical structure of the substance does not contain metals or metalloids and the experience in handling shows that the substance does not react with water.

8.11.2 Comparison with the CLP criteria

Since the chemical structure of the substance does not contain metals or metalloids and since the experience in handling shows that the substance does not react with water, the classification procedure for this class need not be applied to Pyraclostrobin.

8.11.3 Conclusion on classification and labelling for self-heating substances

Pyraclostrobin is not to be classified as substance, which in contact with water emits flammable gases.

8.12 Oxidising liquids

Hazard class not applicable.

8.13 Oxidising solids

Table 12: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EC A 17	The study of oxidizing properties indicates that the active substance is not considered to be an oxidizing agent.		CHE2000-464: Loeffler, 1998

Method	Results	Remarks	Reference
EC A 17	Pyraclostrobin technical (solidified melt) is not an oxidising substance.		2679557: Achhammer, 2013
EC A 17	Pyraclostrobin technical (crystalline) is not an oxidising substance.		2679549: Loehr, 2011 2679554: Loehr, 2011

8.13.1 Short summary and overall relevance of the provided information on oxidising solids

The study on oxidizing properties according to test method EC A.17 indicates that the active substance is not considered an oxidizing agent.

8.13.2 Comparison with the CLP criteria

Results from method EC A.17 are not sufficient to conclude on oxidising properties. Since no UN test O.1 or O.3 was conducted, the available data are not sufficient for the assessment of the oxidising properties (see CLP Annex I, 2.14.2.1.).

8.13.3 Conclusion on classification and labelling for oxidising solids

Data not sufficient for conclusion/classification.

8.14 Organic peroxides

Hazard class not applicable: The substance does not contain the peroxide group (-O-O-).

8.15 Corrosive to metals

Hazard class not applicable: Only solids with a melting point below 55 °C need to be tested. Pyraclostrobin does not fulfil this criterion.

8.16 Desensitised explosives

Data lacking.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Pyraclostrobin is an active substance in plant protection products that is not currently listed in Annex VI of Regulation (EC) No 1272/2008 for physical hazards. The Dossier Submitter (DS) proposed no classification for all physical hazards. The substance is solid which means

that hazard classes related to gases and liquids are not relevant for its physical hazard classification.

Explosives

Pyraclostrobin was tested for explosive properties using EC Method A.14 and was found not to be explosive. However, the method EC A.14 is not sufficient on its own to conclude on explosive properties. The differential scanning calorimetry (DCS) result shows an energy release over 1200 J/g. Pyraclostrobin contains functional groups associated with explosive properties (Table A6.1 in Appendix 6 of the UN RTDG) and the calculated oxygen balance is not less than -200. Therefore, it cannot be excluded that the substance might have explosive properties.

According to the CLP Regulation (Annex I, 2.1.2.3.), explosive properties are tested using UN test series 2 to 8. Corresponding UN test results were not available.

The DS concluded that data are not sufficient for concluding on classification.

Flammable solids

Three studies on flammability performed according to EC A.10 test showed that pyraclostrobin is not highly flammable. Substance did not burn under test conditions. Since the available data from an A.10 test method indicate that a classification as a flammable solid does not apply (result: not highly flammable), no more testing is necessary (ECHA Guidance, Chapter R.7a: Endpoint specific guidance, R.7.1.10.3). As a conclusion, the DS proposed no classification of pyraclostrobin.

Self-reactive substances

Pyraclostrobin contains functional groups which are associated with explosive properties (Table A6.1 in Appendix 6 of UN RTDG) and functional groups indicating self-reactive properties (Table A6.3 in Annex 6 of UN RTDG).

According to CLP Regulation, self-reactive properties are tested using UN test series A to H; the hazard class can be assessed also based on the criteria in CLP Annex I, 2.8.4.2 (waiver). Since no corresponding UN test results are available and the substance contains above mentioned groups, it cannot be excluded that the substance has self-reactive properties.

DS concluded that data are not sufficient for concluding on classification.

Pyrophosphoric solids

No studies are available. Based on practical experience in handling the substance, pyraclostrobin does not ignite spontaneously on coming into contact with air at normal temperatures. No classification was proposed by the DS.

Self-heating substances

Two studies conducted in accordance with EC A. 16 are available. In these studies, pyraclostrobin did not self-ignite up to 400°C. Thus, classification as a self-heating for the

substance does not apply. However, the results from this study are not conclusive to assess this hazard class.

According to CLP Regulation, self-heating properties are tested using UN test N.4. No corresponding UN test results are available.

Therefore, DS concluded that data are not sufficient for classification.

Substances which in contact with water emit flammable gases

The chemical structure of the substance does not contain metals or metalloids and, based on experience in handling showing that the substance does not react with water. As a conclusion, the DS proposed no classification of pyraclostrobin.

Oxidising solids

Provided studies performed according to test method EC A.17 indicated that pyraclostrobin is not considered an oxidising substance. However, results from method EC A.17 are not sufficient to conclude on oxidising properties. Since no UN test O.1 or O.3 was conducted, the available data are not sufficient for the assessment of the oxidising properties (CLP Regulation, Annex I, 2.14.2.1.). Therefore, DS concluded that data are not sufficient for classification.

Organic peroxides

Hazard class not applicable for pyraclostrobin as the substance does not contain the peroxide group (-O-O-).

Corrosive to metals

Hazard class not applicable as only solids with a melting point below 55 °C need to be tested. Pyraclostrobin does not fulfill this criterion.

Desensitised explosives

Due to lack of data the hazard class was not assessed by DS but was open for consultation.

Comments received during consultation

Member State Competent Authorities (MSCA) and a company-manufacturer provided comments.

MS provided editorial comments. In the view of the MS a DSC (differential scanning calorimetry) measurement should be performed to confirm the absence of classification of pyraclostrobin as self-reactive substance. DS commented that a new study has been performed by Company-Manufacturer but is not available to the DS. Regarding self-heating substances, the MS was of the opinion that the justification "*as the melting point of the substance is below 160°C, no further test is needed for classification according to CLP guidance*" should be added. DS agreed and indicated that a new study has been performed by Company-Manufacturer but is not available to DS.

The Company-Manufacturer provided the results of the new studies for some hazard classes and the opinion regarding the proposed classification.

- Explosives: The new study (BASF DocID 2020/2027396) using required UN test series indicated that substance is not considered to exhibit a danger of explosion. The provided data (method used and results) are presented in the section Additional key elements. The results are not leading to a classification according to GHS and transport classification class 1.
- Self-reactive substances: In the new study (BASF DocID 2020/2027397) using UN test H.4 the SADT (self-accelerated decomposition temperature) was determined to be higher than 75°C. According to CLP Annex I, 2.8.4.2, the classification procedures for self-reactive substances and mixtures need not to be applied if the SADT is greater than 75°C. Consequently, pyraclostrobin does not need to be classified as a self-reactive substance.
- Self-heating substances: The new study (BASF DocID 2020/2027396) reports the following results determined in a Grewer oven screening test: The sample showed a self-heating at 103°C (47°C). But the wire basket was empty after the test and the melting point is appr. 70°C, which is below 160°C. Therefore, the UN N.4 test was omitted. Pyraclostrobin does not need to be classified as self-heating substance.
- Oxidising solids: In the new study (BASF DocID 2020/2027396) using UN test method O.3 the mean burning rate of the sample-mixtures is less than the mean burning rate of the reference mixture. Due to these test results, pyraclostrobin does not need to be classified as oxidizing solid according to GHS and transport classification class 5.1.
- Desensitised explosives: This hazard class is not relevant, because pyraclostrobin is not required to be classified as an explosive.

The DS did not provide any response in regard to the new studies as they were not available to them.

Additional key elements

During the process of the preparation of the first draft opinion, RAC requested the new studies mentioned in the public consultation by the Company-Manufacturer. These (BASF DocID 2020/2027396 and 2020/2027397) were provided in September 2022. In the studies, all the tests were carried out based on the methods referred to in Part 2 of Annex I to CLP (UN RTGD, Manual of Tests and Criteria). The study (BASF DocID 2020/2027396) is also in compliance with GLP.

The following tests were submitted:

- **Explosive properties**

A Thermal Stability Differential Scanning Calorimetry (DSC) screening test was performed according to DIN 51005 and OECD TG 113. The sample showed a multi-stage exothermic

reaction (onset-temperature 145°C, energy release 1490 J/g) indicating the need for the following further tests.

The tests were performed according to UN Manual of Dangerous Goods, Manual of Tests and Criteria, Class 1, Regulation EC No. 440/2008 Method A.14, Explosive properties. The following guidelines also apply to the results: EPA Test Guideline OPPTS 830.6316 (1996): Explodability. The method provides a scheme of testing to determine whether a substance presents a danger to explosion.

The question "It is an explosive substance?" is answered based on the results of three tests to assess possible explosive effects. The three tests are:

Test 1 (a): a shock test to determine the ability of the substance to propagate a detonation.

Test 1 (b): a test to determine the effect of heating under confinement.

Test 1 (c): a test to determine the effect of ignition under confinement.

To answer the question "Is the substance too insensitive for inclusion in Class 1?" in general the basic apparatuses used is the same as that for the first question but with less stringent criteria.

Test 2 (a): a shock test to determine sensitivity to shock.

Test 2 (b): a test to determine the effect of heating under confinement.

Test 2 (c): a test to determine the effect of ignition under confinement.

For safety reasons the sensitivity to impact and friction is tested in a preliminary test.

Preliminary tests on on mechanical sensitivity:

Impact sensitivity (3(a)): no explosion, no flames

Friction sensitivity (3(b)): no explosion, no crepitation, no flames

Main Tests:

1. **Thermal sensitivity (E.1)**

The limiting diameter, i.e., the largest diameter of the orifice at which "explosion" occurs, is 1.0 mm.

Assessment of the result within the meaning of Test E.1 (related to Division 4.1): The effect of intensively heating the substance under high confinement is low.

Assessment of the result within the meaning of Test 1 (b): There is an effect from intensively heating the substance under high confinement ("+").

Assessment of the result within the meaning of Test 2 (b): There is no violent effect from intensively heating the substance under high confinement ("-").

2. **Time-pressure-test (C.1; Test 1(c); Test 2(c))**

The test was carried out three times. A gauge pressure of 2070 kPa was not reached in any trial.

Assessment of the result within the meaning of Test 1(c): The maximum pressure reached in any one test is less than 2070 kPa gauge. The substance shows not the ability to deflagrate ("-").

Assessment of the result within the meaning of Test 2(c): The time for a pressure-rise from 670 kPa to 2070 kPa is 30 ms or more or a pressure of 2070 kPa gauge is not reached. The substance shows slow or no deflagration ("-").

3. **Ability to propagate a detonation or sensitivity to shock**

BAM Trauzl test (F.3)

The substance has "No" explosive power.

UN GAP test (A.5; Test 1(a); Test 2(a))

Test was omitted due to result of preliminary test (BAM Trauzl test, F.3).

- **Flammable solids**

Test was performed according to the test method described in the UN Manual of Dangerous Goods, Manual of Tests and Criteria, Part III, Test N.1. The sample was tested in its commercial form. In the preliminary screening test the burning time for distance of 200 mm was 135 s, meaning that the preliminary screening test is negative (burning rate > 2 min). The main test was omitted due to result of preliminary test.

- **Relative self-ignition temperature for solids**

Test was performed according to Regulation EC No 440/2008, test method A.16. The sample was tested in its commercial form. No self-heating was detected up to 450°C.

- **Self-heating substances**

Test was performed according to the test method described in the VDI guideline 2263, part 1, chapter 1.4.1, 1990. In Grewer oven screening test, the sample showed a self-heating at 103°C (T rise: 47°C). But the wire basket was empty after the test. Melting point is appr. 70°C, which is below 160°C. Therefor the UN N.4 test was omitted.

- **Oxidising solids**

Test was performed in accordance with the test method described in the UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria, Part III, Test O.3. The test indicated that mean burning rate of the sample-mixtures is less than the mean burning rate of the reference mixture.

- **Pyrophoric solids**

Test for pyrophoric properties of solids (UN test N.2) has not been carried out because the substance is known to be stable at room temperature for prolonged periods of time (days).

- **Heat accumulation storage test**

Test was performed according to UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria, Part II, Test H.4. The SADT (self-accelerated decomposition temperature) was determined to be higher than 75°C.

Assessment and comparison with the classification criteria

During the preparation of the first draft opinion, new studies mentioned in the public consultation by the Company-Manufacturer were provided (BASF DocID 2020/2027396 and 2020/2027397). All the tests were carried out based on the methods referred to in Part 2 of Annex I to CLP (UN RTGD, Manual of Tests and Criteria). The study (BASF DocID 2020/2027396) is also in compliance with GLP.

RAC is of the opinion that it is appropriate to consider data from these new studies for classification of the substance as they were indicated during the Consultation, were received in sufficient time to be fully evaluated and were conducted according to appropriate methods.

Explosives

Pyraclostrobin is not considered to be explosive based on Method EC A.14. However, the results from this study are not conclusive as the method is not totally in line with the CLP Regulation. The negative A.14 test provides supporting information that pyraclostrobin is not explosive; however, it is not sufficient to conclude on the classification.

Screening procedure (Annex I, section 2.1.4.3 of the CLP Regulation)

Based on the screening procedure applied to pyraclostrobin, the substance does not fulfil any of the conditions set out in CLP Regulation, Annex I, 2.1.4.3, (a-c). Therefore, the substance could be a potential explosive and thus the acceptance procedure has to be performed (CLP Guidance, section 2.1.4.2.).

- Pyraclostrobin has chemical groups which may indicate explosive properties according to Table A6.1 in Appendix 6 of the UN RTDG. Two contiguous nitrogen atoms are present in the pyrazole ring and N-O group.
- Pyraclostrobin contains groups associated with explosive properties which include oxygen and calculated oxygen balance is not less than -200.
- Pyraclostrobin was found to have a measured exothermic decomposition energy of 1490 J/g which is higher than the indicated value of 500 J/g in Table A6.2 of UN MTC and the decomposition onset temperature of the substance is 145°C which is lower than indicated temperature of 500°C in Table A6.2 of UN MTC.

Acceptance procedure (Annex I, Figure 2.1.2 of the CLP Regulation)

Since pyraclostrobin is neither manufactured with a view to using it for practical explosive purposes or pyrotechnic effects, nor is it a candidate for ammonium nitrate emulsion, suspension or gel, the first question refers to the basic explosive properties investigated in Test series number 1. This test line-up answers the question "*Is it an explosive substance/mixture?*" based on three assays:

- Type 1 (a): a shock test with defined booster and confinement to determine the ability of the substance to propagate a detonation (*UN Gap test*, zero gap);
- Type 1 (b): a test to determine the effect of heating under confinement (Koenen test);

- Type 1 (c): a test to determine the effect of ignition under confinement (Time/pressure test).

Pyraclostrobin was not subjected to type 1(a) test (UN Gap test, zero gap*); however, according to the paragraph 2.1.4.2 of CLP Regulation, if the exothermic decomposition energy of organic materials is 800 J/g or more, tests 1 (a) and 2 (a) need not be performed if the outcome of the ballistic mortar Mk.IIId test (F.1), or the ballistic mortar test (F.2) or the BAM Trauzl test (F.3) with initiation by a standard No 8 detonator (Appendix 1 to the UN RTDG, Manual of Tests and Criteria) is 'no'. In this case, the results of test 1 (a) and 2 (a) are deemed to be '-'.* Since pyraclostrobin has an exothermic decomposition energy of 1490 J/g and result of the BAM Trauzl test (F.3) is 'no', waiving this test was appropriate.

Pyraclostrobin was positive in a Koenen test and negative in a time/pressure test. *According to paragraph 2.1.4.5.1 of CLP guidance, the question is answered 'Yes' if a '+' is obtained in any of the three types of tests.* As the Koenen test has shown a positive conclusion the line of decision has to be continued.

The next question that has to be answered is the following: "Is the substance/mixture too insensitive for acceptance into this Class?" The response is given following the results obtained in Test Series 2. This battery of assays comprises the same tests as the Series 1 but with less stringent criteria.

- Type 2 (a): a shock test with defined initiation system and confinement to determine sensitivity to shock (UN gap test) (with a defined gap e.g., 50 mm);
- Type 2 (b): a test to determine the effect of heating under confinement (Koenen test);
- Type 2 (c): a test to determine the effect of ignition under confinement (Time/pressure test).

The UN gap test is waived for pyraclostrobin as shown before. Pyraclostrobin was negative in a Koenen test and time/pressure test. *According to paragraph 2.1.4.5.1 of CLP guidance, if the answer is 'Yes', the substance is rejected from this class; it is not an explosive.*

Overall, based on Test Series 1 and Test Series 2 of the decision logic the pyraclostrobin does not meet the classification criteria for classification as explosive.

According to the CLP Guidance (paragraph 2.1.4.5.1), it is recommended to carry out Test Series 3 before Test Series 1 and 2 for safety reasons due to the small sample amount needed. It is also recommended to carry out Test Series 3 even if negative results have been obtained in Test Series 1 and/or 2 because only Test Series 3 gives information about the thermal stability and the sensitivity to mechanical stimuli (impact and friction). Test Series 3 is used to answer the questions 'Is the substance/mixture thermally stable?' and 'Is the substance/mixture too dangerous for transport in the form in which it was tested?' This involves tests for determining the sensitiveness of the substance or mixture to mechanical stimuli (impact and friction), and to heat and flame. The following four types of tests are used (recommended test is indicated within brackets):

- Type 3 (a): a falling weight test to determine sensitiveness to impact (BAM Fallhammer);
- Type 3 (b): a friction; or impacted friction test to determine sensitiveness to friction (BAM friction apparatus);

- Type 3 (c): an elevated temperature test to determine thermal stability (thermal stability test at 75 °C);
- Type 3 (d): an ignition test to determine the response of a substance or mixture to fire (small scale burning test).

Based on results from preliminary tests on mechanical sensitivity, impact sensitivity (3(a)) and friction sensitivity (3(b)), pyraclostrobin successfully passed the Type 3(a) and Type 3(b) tests. The results for Type 3 (c) and Type 3 (d) tests are not available.

In conclusion, RAC is of the opinion that based on applied acceptance procedure (CLP Regulation, Annex I, Figure 2.1.2) and supported by negative EU A.14 test **no classification for explosives is warranted for pyraclostrobin**.

Flammable solids

Pyraclostrobin was tested for flammability using UN test N.1 which has demonstrated that the substance is not flammable. The results of the experimental test do not fulfil the criteria in CLP Regulation, Table 2.7.1.

Pyraclostrobin was not considered to be highly flammable in an experimental study performed according to method EU A.10. RAC notes that the result "not highly flammable" complies with the ECHA guidance on information requirements and chemical safety assessment (R.7.1.10.3), wherein it is stated that data from an A.10 test method indicate that a classification as a flammable solid does not apply (result: not highly flammable), no more testing is necessary.

Overall, RAC is of the opinion that **no classification for flammable solids is warranted for pyraclostrobin**.

Self-reactive substances

Based on the screening procedure (CLP Regulation, Annex I, 2.8.4.2) applied to pyraclostrobin, the substance has chemical groups which may indicate explosive properties according to Table A6.1 in Appendix 6 of the UN RTDG. Two contiguous nitrogen atoms are present in the pyrazole ring and N-O group. In addition, the substance also has chemical groups associated with self-reactive properties according to Table A6.3 in Appendix 6 of the UN RTDG. Two phenyl groups are present (unsaturation, olefins).

Pyraclostrobin was tested for self-reactive substance using UN test H.4 which has shown that SADT [self-accelerating decomposition temperature] was higher than 75°C. Result of this study support no classification of pyraclostrobin as a self-reactive substance as the substance fulfills the condition set out in CLP Regulation, Annex I, 2.8.4.2, b.

RAC is of the opinion that **no classification for self-reactive substance is warranted for pyraclostrobin**.

Pyrophoric solids

According to classification considerations in CLP Regulation Annex I, 2.10.4.1., the classification procedure for pyrophoric solids need not be applied when experience in manufacture or handling shows that the substance or mixture does not ignite spontaneously on coming into contact with air at normal temperature. The substance is

known to be stable at room temperature for prolonged periods of time (days). Thus, RAC agrees with the DS proposal **not to classify pyraclostrobin as a pyrophoric solid**.

Self-heating substances

EC A.16 tests showed no self-ignition below the melting point. However, a study conducted in accordance with EC A.16 is not sufficient to conclude on the classification according to CLP guidance (2.11.4.2.). The negative A.16 test provides supporting information that pyraclostrobin is not self-heating substance.

The screening procedure applied to pyraclostrobin has shown that the substance fulfils the condition set out in CLP guidance, section 2.11.4.2.:

- Pyraclostrobin has a melting point in the range 63.7 °C – 65.2 °C)/appr. 70°C (submitted study) which is below the 160 °C.
- Pyraclostrobin was completely molten at 160°C (data from submitted study).

Results from the Grewer oven screening test indicated that the sample showed self-heating at 103°C but the wire basket was empty after the test. The substance was molten at appr. 70°C.

Pyraclostrobin was not subjected to UN Test N.4; however, *according to the paragraph 2.11.4.4.1 of CLP guidance (version 5, July 2017), the test procedure need not be applied if the substance or mixture is completely molten at 160 °C.* Since pyraclostrobin has the melting temperature in the range 63.7 °C – 65.2 °C (CLP report)/appr. 70°C (submitted study) which is considerably lower than 160 °C, waiving this test was appropriate. Waiving of the test was supported also by one commenting MS and DS in the public consultation.

RAC is of the opinion that no classification for self-heating substances is warranted for pyraclostrobin based on screening procedure (2.11.4.2., CLP guidance (melting point)) and supported by a negative A.16 test.

Substances which in contact with water emit flammable gases

Based on the screening procedure applied to pyraclostrobin, the substance fulfills all criteria as set out in CLP Regulation, Annex I, 2.12.4.1, a-c. Consequently, the test can be waived (CLP guidance, 2.12.4.2.).

- Pyraclostrobin does not contain metals or metalloid.
- Experience in production and handling shows that the substance does not react with water.
- Pyraclostrobin is soluble in water (1.9±0.17 mg/L).

RAC agrees with the DS **not to classify pyraclostrobin as a substance which in contact with water emit flammable gases.**

Oxidising solids

Screening procedure (Annex I, section 2.14.4.1 of the CLP Regulation)

Based on the screening procedure applied to pyraclostrobin, the substance does not fulfil any of the conditions set out in the CLP regulation, Annex I, 2.14.4.1, a-b. Therefore, the

substance could be regarded as potentially oxidising and thus further testing is required (CLP guidance, section 2.14.4.1.1.).

- Pyraclostrobin contains oxygen and chlorine.
- Pyraclostrobin contains oxygen and chlorine, and the oxygen is chemically bonded to other (nitrogen) than carbon or hydrogen.

Testing requirements (CLP guidance, section 2.14.4.3.)

According to CLP guidance, 2.14.4.1.1 if the substance is 'potentially oxidising' the further testing is required. It is not possible to assign a hazard category on the basis of a theoretical evaluation (based on composition and chemical structure).

The result from the UN MTC Test O.3 (Gravimetric test for oxidizing solids) has shown test has shown that pyraclostrobin is not an oxidizing solid, as the mean burning rate of the sample-mixtures was less than the mean burning rate of the reference mixture. The result of this study supports no classification of pyraclostrobin as an oxidizing solid as the conditions set out in Table 2.14.1 of the CLP regulation are not fulfilled.

Pyraclostrobin had no oxidizing properties according to EC A.17., however this test is not considered in the CLP criteria for this purpose and thus is not sufficient to conclude that the substance is not oxidising (results can be regarded as inconclusive). However, it provides supporting information that pyraclostrobin is not oxidizing solid.

RAC is of the opinion that **no classification for oxidising solids is warranted** for pyraclostrobin based the results of UN O.3 test and supported by negative EC A.17 test.

Organic peroxides

RAC agrees with DS that this hazard class is not applicable to pyraclostrobin as the substance does not contain the peroxide group (-O-O-).

Corrosive to metals

RAC agrees with the DS **not to classify pyraclostrobin as corrosive**. According to the CLP Guidance, section 2.16.4.1. only solids having a melting point lower than 55 °C (test temperature required in UN Test C.1) must be taken into consideration. No corrosiveness to metals is expected for pyraclostrobin as its melting point is 63.7 °C – 65.2 °C which is above 55 °C.

Desensitised explosive

Pyraclostrobin fulfils the condition set out in CLP Regulation, Annex I, 2.17.4.1, (a), thus the classification procedure for desensitised explosives does not apply.

- Pyraclostrobin contains no explosives according to the criteria in CLP Regulation, Annex I, Section 2.1.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 13: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
ADE study OECD 417, Wistar rats GLP-compliant; no deviations	Following single oral administration of ^{14}C-BAS 500 F (pyraclostrobin) to male and female Wistar rats at dose levels of 5 or 50 mg/kg bw, not more than about 15 % of the applied radioactivity was excreted via the urine, whereas elimination via the faeces accounted for 81-92 % of the dose by 120 h post-dosing. Taking the assumed biliary excretion of approximately 35 % as well as the wide distribution into account, a total oral absorption and bioavailability of 50 % may be assumed for both sexes irrespective of the dose level. Tissue distribution determination revealed highest amounts of radioactivity in the GI tract and the liver. All other tissues had values comparable to or less than the blood and plasma concentrations. There was no evidence of accumulation of pyraclostrobin.	Oral absorption, although incomplete, was rapid and elimination was nearly complete after 120 hours post-dosing with the major part of radioactivity being excreted during the first 48 hours. The described pattern of absorption, distribution and elimination was not significantly altered by differences in dose level, location of the radiolabel, dosing regimen (single or multiple administration), or sex. Biokinetic parameters revealed a monophasic decrease of plasma concentrations following administration of the high dose (50 mg/kg bw) with a terminal half life of about 20 hours. As for the low dose (5 mg/kg bw), the decline was biphasic with initial and terminal half-lives of around 10 and 31-37 hours, respectively.	TOX2000-705: 1998
ADE study OECD 417, NMRI mouse GLP-compliant; no deviations	Radioactive residues of 14C-BAS 500 F was detectable in the systemic circulation, in bone marrow and in liver of mice, two hours after single oral administration of the test substance formulated in olive oil at a target dose level of 300 mg/kg bw.		ASB2017-5506: 2016
Metabolism EEC 87/302, Wistar rats GLP-compliant; no deviations from EEC method	After oral administration to male and female rats, the systemically available portion of pyraclostrobin was rapidly and extensively metabolised to a large number of biotransformation products accounting mostly for only a small percentage of the applied dose. N-demethoxylation was the quantitatively most important pathway. Phase I biotransformation is further characterised by various hydroxylations, cleavage of the ether bond and further oxidation of the two resulting molecule parts. Combinations of these reactions and the conjugation of the resulting OH-groups with glucuronic acid or sulphate led to the large number of observed metabolites. No major differences were observed with regard to sex and dose level.		TOX2000-708: 1999

Method	Results	Remarks	Reference
Comparative *in vitro* metabolism No guideline available GLP-compliant	This comparative *in vitro* study revealed a lot of similarities but also remarkable difference in metabolism of pyraclostrobin between the species investigated. Cytotoxicity was also different. At the maximum concentration of 10 µM, human liver cells proved more vulnerable than those from rats and rabbits. At a lower concentration of 3 µM, the difference virtually disappeared. Metabolism of pyraclostrobin appeared faster in cells and microsome preparations obtained from rabbits and rats as compared to those from humans and was likely slowest when dog microsomes were taken into consideration. Most efficient metabolism was noted in rabbits. Some metabolites such as 500M04, 500M108, 500M103, 500M104 and 500M88 were common to all test species even though there may be quantitative differences and the site of radiolabelling might also have an impact. The study did not elucidate any metabolite as unique to human samples in that way that it was not found either in rabbit or rat hepatocytes. From a qualitative point of view, the rabbit metabolite pattern is most similar to the human one. In rat hepatocytes, in contrast, hydroxylation and conjugation to 500M104 seems to be more pronounced as compared to humans and rabbits. The investigations with microsomes confirmed the picture obtained with hepatocytes.	In rat samples, a few metabolites could not be identified that were abundant in the experiments with human and rabbit samples. An explanation might be that cleavage of the amide bond resulting in the formation of the metabolite 500M106 is a major degradation pathway in human and rabbit cells. Subsequently, 500M106 is further metabolised by conjugation with glucuronic acid to 500M107. In human and rabbit samples, the metabolite 500M02, which is formed by dimerization, was also identified. In preparations from dogs, trace amounts of 500M02 were also found.	ASB2015-8295: Funk et al., 2014

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

Following single oral administration of ^{14}C-radiolabeled pyraclostrobin at doses of 5 or of 50 mg/kg body weight (bw) to rats, oral absorption was rapid but incomplete. At 120 h post-dosing, only 15 % of the applied radioactivity was excreted via the urine, whereas elimination via the faeces accounted for 80-90 % of the dose. However, irrespective of the dose level, a total bioavailability of 50 % may be assumed for both sexes when the biliary excretion of approximately 35 % (determined at 48 h post-dosing) and the wide distribution to organs and tissues are taken into account. Pharmacokinetic parameters revealed a monophasic decrease of plasma concentrations following administration of the high dose with a half-life of about 20 hours. Subsequent to low dose application, the decline was biphasic with initial and terminal half-lives of around 10 and 31 to 37 hours, respectively (TOX2000-705: 1998).

Tissue distribution determination revealed highest amounts of radioactivity in the GI tract followed by the liver. All other tissues had values comparable to or less than the plasma concentrations.

There was no evidence of accumulation of pyraclostrobin (TOX2000-705: 1998).

The systemically available portion of pyraclostrobin was rapidly and extensively metabolised. N-desmethoxylation was the quantitatively most important pathway. Phase I biotransformation is further characterised by various hydroxylations, cleavage of the ether bond and further oxidation of the two resulting molecule parts. Combinations of these reactions and the conjugation of the resulting OH-groups with glucuronic acid or sulphate led to a large number of observed metabolites with most of them accounting for only a small percentage of the applied dose (TOX2000-708: 1999, ASB2017-5452: 2014 and ASB2015-8294: 2014).

This pattern of absorption, distribution, metabolism and elimination is not significantly altered by dose level, site of radiolabel, dosing regimen (single or multiple administration) or sex.

A comparative *in vitro* study in hepatocytes of human origin and in rat, dog and rabbit liver cells (ASB2015-8295: Funk et al., 2014) revealed a lot of similarities but also remarkable difference in metabolism. Metabolism of pyraclostrobin appeared faster in cells and microsome preparations obtained from rabbits and rats as compared to those from humans. It was likely the slowest when dog microsomes were taken into consideration. Most efficient metabolism was noted in rabbits. Some metabolites such as 500M04, 500M108, 500M103, 500M104 and 500M88 were common to all test species even though there may be quantitative differences. The study did not elucidate any metabolite as unique to human samples in that way that it was not found either in rabbit or rat hepatocytes. From a qualitative point of view, the rabbit metabolite pattern appeared most similar to that observed in human cells. In rat samples, in contrast, a few metabolites such as 500M02 or 500M106 were not detected that were abundant in the experiments with human and rabbit samples.

It should be brought up that, as already mentioned above, pyraclostrobin, once absorbed into systemic circulation, is extensively metabolised via various biotransformation reactions (e.g. N-desmethoxylation, hydroxylation, cleavage of the ether bond), which can yield numerous metabolites. For the toxicological evaluation of the potential metabolites of pyraclostrobin, the metabolites were categorised into 7 groups based on their structural similarities (e.g. using the Tanimoto coefficient). Several toxicity studies (focused mostly on genotoxicity) have been conducted on a number of metabolites such as 500M04, 500M24, 500M106, 500M02, and the results of these studies were then considered for the evaluation of the other metabolites in the respective groups. As these studies are specifically related to the metabolites of pyraclostrobin and not the parent substance itself, they are considered outside of the scope for CLP classification of pyraclostrobin and are not included here in this CLH dossier.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity

10.1.1 Acute toxicity - oral route

Table 14: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD50	Reference
Acute oral toxicity OECD 401 GLP-compliant No deviations	Rat (Wistar) M/F 5/sex/dose	Pyraclostrobin (purity: 98.5 %)	2000 and 5000 mg/kg bw Single dose Observed up to 15-d post-dosing	LD_{50} > 5000 mg/kg bw No deaths but clinical signs at both dose levels	TOX2000-709: 1998

Table 15: Summary table of human data on acute oral toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
N/A				

Table 16: Summary table of other studies relevant for acute oral toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Dose range-finding study for in vivo micronucleus test in mice	Pyraclostrobin (purity 98.2%) 125, 250, 300, 400, 500, 1000, 2000 mg/kg bw	Test material was formulated in olive oil (10 mL/kg bw). Single oral administration Treated mice (NMRI; both males and females) were observed for a period of up to 5 days after administration.	Acute LD_{50} values: 449, 453 and 451 mg/kg bw (for male mice, female mice and combined sexes, respectively). Clinical signs (piloerection and hunched posture) were seen at dose levels of 250 mg/kg bw and higher. At higher doses further clinical signs were reported.	ASB2017-5505: 2016

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

In an acute oral toxicity study conducted in accordance with OECD Guideline 401 (TOX2000-709: 1998), there was no mortality observed in Wistar rats of either sex exposed to a single dose of 2000 or 5000 mg/kg bw of pyraclostrobin. Clinical signs such as dyspnoea, apathy, staggering, piloerection and diarrhoea were noted in both sexes at the low and high dose levels, but they disappeared within a few days after administration. The oral LD_{50} was set at > 5000 mg/kg bw for rats.

Even though the abovementioned rat study does not fulfil the requirements for classification and labelling, data obtained in NMRI mice suggest a markedly higher toxicity of pyraclostrobin in this species. In a dose range-finding experiment (tested from 125 to 2000 mg/kg bw) for a mouse bone marrow micronucleus assay with oral administration (ASB2017-5505: 2016), an acute LD_{50} of approximately 450 mg/kg bw was calculated for both sexes. Clinical signs (piloerection and hunched posture) were seen at dose levels of 250 mg/kg bw and higher. At higher doses (≥ 400 mg/kg bw), further clinical signs (e.g. reduced general state, salutatory spasm, irregular respiration) were reported. Below is the mortality data from this dose range-finding study.

Table 17: Mortality data from the dose range-finding experiment of the mouse bone marrow micronucleus assay (ASB2017-5505: 2016)

Dose level [mg/kg bw]	Animals treated (M/F)	Dead animals (M/F; time of death)
2000	2/2	2/2 (within 15 min)
1000	4/4	4/4 (15 min to 1 d)
500	4/4	3/3 (30 min to 2 d)
400	5/5	1/1 (1 d)
300	5/5	0/0
250	5/5	0/0
125	5/5	0/0

10.1.1.2 Comparison with the CLP criteria

Table 18: Comparison with the CLP criteria

Toxicological result (pyraclostrobin)	CLP criteria (Proposed classification in bold)
Oral LD_{50}, rat: >5000 mg/kg (m/f) Oral LD_{50}, mouse: ~450 mg/kg (m/f)	Cat. 4 (H302): $300 < LD_{50} \leq 2000$ mg/kg (oral) Cat. 3 (H301): $50 < LD_{50} \leq 300$ mg/kg (oral) Cat. 2 (H300): $5 < LD_{50} \leq 50$ mg/kg (oral) Cat. 1 (H300): $LD_{50} \leq 5$ mg/kg (oral)

10.1.1.3 Conclusion on classification and labelling for acute oral toxicity

Acute oral toxicity: Category 4, "Harmful if swallowed", H302, with an ATE of 450 mg/kg bw.

10.1.2 Acute toxicity - dermal route

Table 19: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Value LD_{50}	Reference
Acute dermal toxicity OECD 402 GLP-compliant No deviation	Rat (Wistar) M/F 5/sex/dose	Pyraclostrobin (purity 98.2%) Semi-occlusive application	2000 mg/kg bw Single exposure for 24 h 14-d post-application observation period	LD_{50} >2000 mg/kg bw, no deaths, no clinical signs except slight local irritation, equivocal reduction in body weight gain in females	TOX2000-710: 1998

Table 20: Summary table of human data on acute dermal toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
N/A				

Table 21: Summary table of other studies relevant for acute dermal toxicity

Type of study/ data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
N/A				

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

In an acute dermal toxicity study conducted in accordance with OECD Guideline 402 (TOX2000-710: 1998), the dermal LD_{50} was found > 2000 mg/kg bw for male and female rats. One day after application, very slight to well-defined erythema, mechanical skin lesion due to adhesive test substance were observed in all animals. No pathological findings were detected in the animals at necropsy.

10.1.2.2 Comparison with the CLP criteria

Table 22: Comparison with the CLP criteria

Toxicological result (pyraclostrobin)	CLP criteria
Dermal LD_{50}, rat: >2000 mg/kg bw (m/f)	Cat. 4 (H312): $1000 < LD_{50} \leq 2000$ mg/kg (dermal) Cat. 3 (H311): $200 < LD_{50} \leq 1000$ mg/kg (dermal) Cat. 2 (H310): $50 < LD_{50} \leq 200$ mg/kg (dermal) Cat. 1 (H310): $LD_{50} \leq 50$ mg/kg (dermal)

10.1.2.3 Conclusion on classification and labelling for acute dermal toxicity

No classification for acute dermal toxicity is warranted. Also, given there was no mortality observed in the acute eye irritation study, the EUH070 statement is not needed.

10.1.3 Acute toxicity - inhalation route

Table 23: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Acute inhalation toxicity OECD 403 GLP-compliant No significant deviations that would affect the study validity.	Rat (Wistar) M/F 5/sex/group	Pyraclostrobin (purity 98.2 %) Liquid aerosol MMAD between 1.0 and 2.9 µm with GSD between 2.5 and 3	0, 0.31, 1.07, 5.3 mg/L air (vehicle: acetone) 4 h, nose only 14-d post-exposure observation period	LC_{50} 0.69 mg/L (calculated) 100 % mortality at the two upper concentrations within 75 minutes of exposure; clinical signs but no deaths at lowest concentration	TOX2000-711: 1997
Acute inhalation toxicity OECD 403 GLP-compliant No deviations	Rat (Wistar) M/F 5/sex/group	Pyraclostrobin (purity 98.2 %) Liquid aerosol MMAD between 1.2 and 1.7 µm with GSD between 2.5 and 2.7	0, 0.52, 0.65, 0.85 mg/L air (vehicle: acetone) 4 h, nose only 14-d post-exposure observation period	LC_{50} 0.58 mg/L (calculated) 100 % mortality at the maximum and 90 % mortality at the intermediate concentration on day of treatment; clinical signs and one death at the low concentration	ASB2008-5020: 2002
Acute inhalation toxicity OECD 403 GLP-compliant No deviations Considered as supplementary data as ~40 % of	Rat (Wistar) M/F 5/sex/group	Formulation containing 38.1% pyraclostrobin in Solvesso Liquid aerosol MMAD between 2.7 and 4.3 µm	Pyraclostrobin in Solvesso (40 %): 0, 0.89, 1.96, 4.07, 7.3 mg/L Calculated as pyraclostrobin: 0.34, 0.75, 1.55 or 2.78 mg/L	Pyraclostrobin in Solvesso (40 %): 4.07 mg/L < LC_{50} > 7.3 mg/L (M) LC_{50} : 5.47 mg/L (F); 90 % mortality at maximum	TOX2001-881: 2001

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
the active substance was tested		with GSD between 2.5 and 2.7	4 h, nose only 14-d post-exposure observation period	concentration; clinical signs but only few deaths (10%) at the two intermediate concentrations Calculated as pyraclostrobin: $1.55 < LC_{50} < 2.78$ mg/L (M) LC_{50}: 2.1 mg/L (F)	

Table 24: Summary table of human data on acute inhalation toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
N/A				

Table 25: Summary table of other studies relevant for acute inhalation toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
N/A				

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

Two studies performed in accordance with OECD Guideline 403 both showed high acute inhalation toxicity of pyraclostrobin in rats exposed nose-only to this test substance as a liquid aerosol for 4 hours.

In the earlier study (TOX2000-711: 1997), 100 % mortality was observed at the two upper concentrations of 1.07 and 5.30 mg/L within 75 minutes post-exposure. At the lowest concentration of 0.31 mg/L, no mortality was observed by the end of the 14-day observation period, but clinical examination revealed irregular respiration, bloody nose discharge, piloerection and smeared fur until day 7 post-exposure.

Necropsy of the mid-concentration (1.07 mg/L) animals showed agonal congestive hyperaemia. No further macroscopic pathological findings were noted in animals exposed to the low concentration examined at the end of the study or in the high concentration-exposed animals that died during the study. The LC_{50} from this study was calculated to be 0.69 mg/L.

In the later study (ASB2008-5020: 2002), 100 % mortality was also observed at the highest tested concentration of 0.85 mg/L on the day of exposure. At the intermediate concentration of 0.65 mg/L, 5 out of 5 males and 4 out of 5 females died (90% mortality), and at the lowest concentration of 0.52 mg/L, 1 female died. All deaths occurred on the day of exposure. Clinical signs were observed at all concentrations and consisted of visually accelerated respiration, attempts to escape, squatting posture and piloerection, but these signs had resolved by day 7 of the observation period at the latest.

Gross necropsy of the animals that died during the study revealed mainly red discolorations of the lungs. In most cases, all lung lobes were affected. Additionally, wet and contaminated fur was observed in animals at the mid-concentration level that died during the study. One intermediate concentration male also displayed lung oedema in all lobes. The LC_{50} from this later study was calculated to be 0.58 mg/L.

Overall, the calculated inhalative LC_{50} values for the two studies are similar (0.58-0.69 mg/L) and justify the classification as Acute Tox., Cat. 3, H331 for pyraclostrobin.

A supplementary acute inhalation toxicity study revealed higher calculated rat LC_{50} value of pyraclostrobin when tested as a formulation containing ~40 % of pyraclostrobin.

10.1.3.2 Comparison with the CLP criteria

Table 26: Comparison with the CLP criteria

Toxicological result (pyraclostrobin)	CLP criteria(Proposed classification in bold)	
Inhalation LC_{50}, rat: 0.58-0.69 mg/L (nose-only, mist/aerosol, 4 h)	No classification:	> 20 mg/L (vapours)
		> 5 mg/L (dusts/mist)
	Cat. 4 (H332):	$10.0 < LC_{50} \leq 20.0$ mg/L (vapours)
		$1.0 < LC_{50} \leq 5.0$ mg/L (dusts/mists)
	Cat. 3 (H331):	$2.0 < LC_{50} \leq 10.0$ mg/L (vapours)
		$0.5 < LC_{50} \leq 1.0$ mg/L (dusts/mists)
	Cat. 2 (H330):	$0.5 < LC_{50} \leq 2.0$ mg/L (vapours)
		$0.05 < LC_{50} \leq 0.5$ mg/L (dusts/mists)
	Cat. 1 (H330):	$LC_{50} \leq 0.5$ mg/L (vapours)
		$LC_{50} \leq 0.05$ mg/L (dusts/mists)

10.1.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Acute inhalation toxicity: Category 3, “Toxic if inhaled”, H331, with an ATE of 0.58 mg/L (dusts or mists).

RAC evaluation of acute toxicity

Summary of the Dossier Submitter’s proposal

Acute oral toxicity

No mortality was observed in an acute oral toxicity study (OECD TG 401, vehicle: aqueous tylose) in Wistar rats for pyraclostrobin up to 5000 mg/kg bw. However, the dossier submitter (DS) proposed Acute Tox. 4; H302 for pyraclostrobin for acute oral toxicity based on an LD_{50} of approx. 450 mg/kg bw (the ATE) calculated in a dose-range finding study (vehicle: olive oil) for *in vivo* micronucleus test in NMRI mice.

Acute dermal toxicity

No mortality was observed in an acute dermal toxicity study (OECD TG 402) in Wistar rats for pyraclostrobin up to 2000 mg/kg bw. No other relevant studies were identified by the DS. Therefore, the DS proposed “no classification” for pyraclostrobin for acute dermal toxicity.

Acute inhalation toxicity

In two acute inhalation toxicity studies (OECD TG 403) in Wistar rats for pyraclostrobin (98% purity, liquid aerosol), LC_{50}-values of 0.69 mg/L and 0.58 mg/L, respectively, were obtained. In a third acute inhalation toxicity study (according to OECD TG 403) in Wistar rats for pyraclostrobin (only 40% of the active substance in the test material, liquid aerosol), the calculated LC_{50}-values (for 100% active substance) were >1.55 & <2.78 mg/L for males and 2.1 mg/L for females.

The DS proposed Acute Tox. 3; H331 with an ATE of 0.58 mg/L (dusts or mists) for pyraclostrobin for acute inhalation toxicity.

Comments received during consultation

Three MSCAs and a Company/Manufacturer submitted comments during the consultation. The three MSCAs supported the DS proposal. The Company/manufacturer commented that the higher acute oral toxicity of the lipophilic pyraclostrobin in mice is likely caused by the non-aqueous vehicle (olive oil) enhancing the adsorption in the gastro-intestinal tract. The DS responded that their proposal is appropriate in accordance with the Guidance on the Application of the CLP Criteria (CLP guidance, ECHA, 2017) which states that the classification is based on the lowest ATE available taking into consideration the different species (rat vs mouse) or vehicles used (aqueous tylose vs olive oil).

Assessment and comparison with the classification criteria

Acute oral toxicity

In an acute oral toxicity study (OECD TG 401, GLP-compliant and no deviations) with pyraclostrobin (purity: 98.5%, vehicle: aqueous tylose) in Wistar rats (5/sex/dose), a single oral dose of 2000 or 5000 mg/kg bw resulted in no mortality (observation period: 15-d post dosing). Clinical signs such as dyspnoea, apathy, staggering, piloerection and diarrhoea were noted in both sexes at the low and high dose levels, but they disappeared within a few days after administration (TOX2000-709: 1998).

In a dose-range finding (DRF) study for an *in vivo* micronucleus test in NMRI mice (males (M) and females (F)), pyraclostrobin (purity: 98.2%, vehicle: olive oil) was given at different single oral doses ranging from 125 to 2000 mg/kg bw (observation period: 5 days post dosing). A combined LD_{50} of approx. 450 mg/kg bw was calculated for both sexes. Clinical signs (piloerection and hunched posture) were seen at dose levels of 250 mg/kg bw and higher. At higher doses (≥ 400 mg/kg bw), further clinical signs (e.g., reduced general state, salutatory spasm, irregular respiration) were reported (ASB2017-5505: 2016). The mortality data from this DRF study is reported in the table below.

***Table**: Mortality data from the DRF study (ASB2017-5505: 2016) for in vivo micronucleus test in mice (Table 17 in the CLH report)*

Dose level [mg/kg bw]	Animals treated (M/F)	Dead animals (M/F; time of death)
2000	2/2	2/2 (within 15 min)
1000	4/4	4/4 (15 min to 1 d)
500	4/4	3/3 (30 min to 2 d)

400	5/5	1/1 (1 d)
300	5/5	0/0
250	5/5	0/0
125	5/5	0/0

In the DRF study for *in vivo* micronucleus test in mice, 3 out of 4 animals tested (for each sex) died within 30 minutes to 2 days at 500 mg/kg bw while 1 of 5 animals tested (for each sex) died within 1 day at 400 mg/kg bw. RAC notes that the observation period post-dosing is shorter (5 days) in this study, which raises an uncertainty as to whether a longer, standard (14 days) observation period possibly could have led to higher mortality and thus a lower LD_{50}. Although the DRF study is not a standard acute oral toxicity study, RAC considers the calculated LD_{50} of approx. 450 mg/kg bw for both sexes in this study as appropriate to base the acute oral toxicity classification on. Considering the lipophilic nature of pyraclostrobin (log P_{ow} of 3.99), olive oil is an appropriate vehicle.

Therefore, RAC agrees with the DS and concludes that **pyraclostrobin warrants classification as Acute Tox. 4; H302 with an ATE of 450 mg/kg bw.**

Acute dermal toxicity

In an acute dermal toxicity study (according to OECD TG 402, GLP-compliant and no deviations) with pyraclostrobin (purity: 98.2%, dissolved in a 0.5% aqueous (bidest) Tylose® CB 30,000, semi-occlusive application) in Wistar rats (5/sex), a single exposure for 24 hours of 2000 mg/kg bw resulted in no mortality (observation period: 14 days post application). One day after application, very slight to well-defined erythema, a mechanical skin lesion due to the adhesive nature of the test substance was observed in all animals. No pathological findings were detected in the animals at necropsy (TOX2000-710: 1998). RAC notes that the aqueous vehicle used may not have completely dissolved the lipophilic pyraclostrobin.

Since there were no mortalities at 2000 mg/kg bw in the standard study, RAC agrees with the DS and concludes that **pyraclostrobin warrants no classification for acute dermal toxicity.**

Acute inhalation toxicity

In the first acute inhalation toxicity study (OECD TG 403, GLP-compliant and no significant deviations affecting study validity) with pyraclostrobin (purity: 98.2%, liquid aerosol, mass median aerodynamic diameter (MMAD) between 1.0 and 2.9 µm with a geometric standard deviation (GSD) between 2.5 and 3), Wistar rats (5/sex/group) were exposed nose-only for 4 hours to 0, 0.31, 1.07 or 5.3 mg/L (observation period: 14 days post exposure). There was 100 % mortality at the two highest concentrations within 75 minutes of exposure. Necropsy of the mid-concentration (1.07 mg/L) animals showed agonal congestive hyperaemia. There was no mortality at the lowest concentration (0.31 mg/L) but clinical signs such as irregular respiration, bloody nose discharge, piloerection and smeared fur were observed until day 7 post-exposure. The calculated LC_{50} in this study was 0.69 mg/L (TOX2000-711: 1997).

In the second acute inhalation toxicity study (OECD TG 403, GLP-compliant and no deviations) with pyraclostrobin (purity: 98.2%, liquid aerosol, MMAD between 1.2 and 1.7 µm with GSD between 2.5 and 2.7), Wistar rats (5/sex/group) were exposed nose-only for 4 hours to 0, 0.52, 0.65 or 0.85 mg/L (observation period: 14 days post-exposure). There was 100% mortality at the highest concentration (0.85 mg/L), 90% mortality at the mid-concentration (0.65 mg/L) and 10% mortality at the lowest concentration (0.52 mg/L). All deaths occurred on the day of exposure. Gross necropsy of the animals that died during the study revealed mainly red discolorations of the lungs. In most cases, all lung lobes were affected. Additionally, wet and contaminated fur was observed in animals at the mid-concentration level that died during the study. One mid-concentration male also displayed lung oedema in all lobes. Clinical signs were observed at all concentrations and consisted of visually accelerated respiration, attempts to escape, squatting posture and piloerection, but these signs had resolved by day 7 of the observation period at the latest. The calculated LC_{50} in this study was 0.58 mg/L (ASB2008-5020: 2002).

In the third acute inhalation toxicity study (OECD TG 403, GLP-compliant and no deviation) with pyraclostrobin (formulation containing only 38.1% active substance, liquid aerosol, MMAD between 2.7 and 4.3 µm with GSD between 2.5 and 2.7), Wistar rats (5/sex/group) were exposed nose-only for 4 hours to 0, 0.89, 1.96, 4.07 or 7.3 mg/L (observation period: 14 days post exposure). There was 90% mortality at the highest concentration. Few deaths (10%) and clinical signs were noted at the two mid-concentrations. The calculated LC_{50}-values (for 100% active substance) in this study were >1.55 & <2.78 mg/L for males and 2.1 mg/L for females (TOX2001-881: 2001).

The LC_{50}-values from the first two studies (0.69 and 0.58 mg/L) are in the range for Acute toxicity Category 3 ($0.5 < LC_{50} \leq 1.0$ mg/L (dusts/mists)). The LC_{50}-values in the third study are above the limit for Category 3; however, this study was conducted with a formulation and a higher upper range of MMAD compared to the other two studies. Overall, RAC agrees with the DS and concludes that **pyraclostrobin warrants classification as Acute Tox. 3; H331 with an ATE of 0.58 mg/L (dusts or mists).**

10.2 Skin corrosion/irritation

Table 27: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
Acute dermal irritation/corrosion OECD 404 GLP-compliant No deviations	Rabbit (NZW) M/F 3/sex/group	Pyraclostrobin (purity 98.2 %)	0.5 g (undiluted) 4 h Semi-occlusive Up to 15-d observation period	Irritant - Erythema and oedema observed starting at 1 and 24 h, respectively, after exposure - Mean scores for erythema for the 6 animals: 1.3, 2.0, 2.0, 1.7, 2.0, 2.0	TOX2000-712: 1998

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
				- Mean scores for oedema for the 6 animals: 0.3, 0.7, 0.0, 0.0, 1.0, 0.3 - Effects not completely reversible for 2 animals by 15 d post-exposure	

Table 28: Summary table of human data on skin corrosion/irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
N/A				

Table 29: Summary table of other studies relevant for skin corrosion/irritation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Skin sensitisation OECD 406 Guinea pig (Dunkin-Hartley), F 20/test group 10/control GLP-compliant No deviations	Pyraclostrobin (purity 99.0 %) Induction: 5 % Challenge: 1 % (maximum non-irritant concentration)	A 5% test substance preparation in 1% Tylose CB 30,000 in aqua bidest was chosen for the percutaneous induction that caused discrete to moderate erythema.	Following both intradermal and percutaneous induction, skin irritation was observed in all treated animals.	TOX2000-714: 1998

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

In the skin irritation study on rabbits (TOX2000-712: 1998) performed in accordance with OECD Guideline 404, pyraclostrobin was proven to be irritating (see table below).

Table 30: Skin irritation scores (erythema/oedema) from the dermal irritation rabbit study (TOX2000-712: 1998)

Animal	Time after patch removal						
Number	1 h	24 h	48 h	72 h	8 d	15 d	Mean (24-72 h)
1	2/0	2/1	2/0	0/0	0/0	0/0	1.3/0.3
2	2/0	2/1	2/1	2/0	1/0	0/0	2.0/0.7
3	2/0	2/0	2/0	2/0	3/1	2/1	2.0/0.0
4	2/0	2/0	2/0	1/0	1/0	1/0	1.7/0.0

Animal	Time after patch removal						
Number	1 h	24 h	48 h	72 h	8 d	15 d	Mean (24-72 h)
5	2/0	2/1	2/1	2/1	2/1	0/0	2.0/1.0
6	1/0	2/1	2/0	2/0	2/0	0/0	2.0/0.3

In most rabbits, erythema (and, in some rabbits, also oedema) extended beyond the area of exposure. Skin findings were not reversible within 15 days in two animals (nos. 3 and 4). In one of these rabbits (no. 3) scaling, erythema and oedema extending beyond the area of exposure were, noted whereas in the other rabbit (no. 4), only erythema beyond the area of exposure was observed. The mean skin irritation score (from 24 to 72 hours) for all 6 animals was calculated to be 1.8 for erythema and 0.4 for oedema.

Even though the requirements for Skin Irritation, Cat. 2 (H315) classification according to CLP are not fully met when only the mean scores for erythema and oedema were considered, the irritation findings were not fully reversible in all affected animals within 15 days and, moreover, skin irritation was also noted in a second species (guinea pig) exposed to formulation containing 5 % pyraclostrobin in the maximisation test performed in accordance with OECD Guideline 406.

10.2.2 Comparison with the CLP criteria

Table 31: Comparison with the CLP criteria

Toxicological result (pyraclostrobin)	CLP criteria (Proposed classification/rationale in bold)
Mean erythema and oedema scores (24-72 h): no animal >2.3 Inflammation in 2 rabbits at the end of the observation period Signs of skin irritation in guinea pigs in the skin sensitisation study	Irritating to skin (Category 2, H315): * at least in 2/3 tested animal a positive response of: Mean value of ≥2.3 - ≤4.0 for erythema/eschar or for oedema * inflammation that persists to the end of the observation period in at least 2 animals

10.2.3 Conclusion on classification and labelling for skin corrosion/irritation

Skin irritation: Category 2, “Causes skin irritation”, H315

Considering the not-completely reversible irritation observed in the skin irritation rabbit study and the skin irritation observed in the guinea pig skin sensitisation study, the data warrants the Skin Irrit., Cat. 2, (H315) classification based on a weight-of-evidence approach.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter’s proposal

In an acute dermal irritation/corrosion study (OECD TG 404) in rabbits, none of the 6 animals tested had a mean score of ≥2.3 and ≤4.0 for erythema/eschar or for oedema. However, the DS proposed Skin Irrit. 2 for pyraclostrobin based on skin irritation that was not reversible within 15 days in 2 animals in the OECD TG 404 study and the irritation observed in the skin sensitisation study (OECD TG 406, Guinea pig maximisation test - GPMT) in a weight of evidence approach.

Comments received during consultation

Three MSCAs and a Company/manufacturer submitted comments during the consultation. Two MSCAs supported the DS proposal. One MSCA did not support the proposal noting that in the RAR for pyraclostrobin, it is stated that remaining test material was seen at termination in the two animals in the OECD TG 404 study, and they considered that this most likely induced mechanical irritation. This MSCA and the company, which also did not support the DS proposal, cautioned on the use of irritation data from the GPMT due to differences in exposure of the substance compared to OECD TG 404 and the use of an adjuvant. The company also commented that according to the CLP guidance (2017), classification for irritation is applicable if at least 4 out of 6 rabbits show a mean score of ≥2.3 and ≤4.0 for erythema/eschar or for oedema. The company considered that the same numerical criteria (4 of 6 animals) should apply to the non-reversibility of skin irritation at the end of the observation period.

In response, the DS noted that in the OECD TG 404 study, remaining test material was found on all rabbits until 8 days post application, but some rabbits recovered by 72 hours even though the test material was remaining. They also noted that the study authors explicitly reported any mechanical irritation observed and that this was only seen in one rabbit up to 24 hours post application. Thus, the DS considered that the irritation findings are most likely due to the chemical nature of the test material. The DS also mentioned that the authors of the OECD TG 404 study concluded that pyraclostrobin was a skin irritant.

The DS noted that the CLP guidance (2017) does not specify any numerical threshold of animals for non-reversibility of skin lesions for studies with more than 3 animals and did not agree with the company. In support of this the DS cited the CLP guidance (2017). According to section 3.2.2.3.2.2 of the CLP guidance (2017), the irritation criteria for classification are fulfilled if

- "a limited degree of alopecia, hyperkeratosis, hyperplasia and scaling occurs. Two animals showing this response are sufficient for the classification as irritant."
- "very elevated mean scores throughout the study are revealed, including lesions persisting at the end of an observation period of normally 14 days. One animal showing this response throughout and at the end of observation period is sufficient for the classification as irritant".

The DS acknowledged that the experimental design of the GPMT is not directly comparable to the OECD TG 404 study and that intradermal injection of an adjuvant can trigger irritating effects. However, the DS noted that in the pre-test of the GPMT the maximum non-irritant concentration of pyraclostrobin was 1% in the two 24-h percutaneous occlusive applications implying that pyraclostrobin might be a potent skin irritant and exposure to higher concentration for a shorter period (e.g., 4 hours) could also lead to skin irritation. The DS considered this information as supporting evidence for classification purposes.

Assessment and comparison with the classification criteria

In the acute dermal irritation/corrosion study (OECD TG 404, GLP-compliant and no deviations), 0.5 g of pyraclostrobin (purity: 98.2%) was applied (semi-occlusive) to 3

rabbits/sex for 4 hours (observation period: 15 days post application). For none of the animals was the mean score (of 24-, 48- and 72-h values) ≥2.3 and ≤4.0 for erythema/eschar or for oedema. See the table below. In most rabbits, erythema (and in some rabbits also oedema) extended beyond the area of exposure. Skin findings were not reversible within 15 days in 2 animals (nos. 3 and 4). In 1 of these rabbits (no. 3) scaling, erythema and oedema extending beyond the area of exposure were noted whereas in the other rabbit (no. 4), only erythema beyond the area of exposure was observed (TOX2000-712: 1998).

***Table**: Skin irritation scores (erythema/oedema) from the OECD TG 404 study (TOX2000-712: 1998) (Table 30 in the CLH report)*

Animal Number	Time after patch removal						Mean (24-72 h)
	1 h	24 h	48 h	72 h	8 d	15 d	
1	2/0	2/1	2/0	0/0	0/0	0/0	1.3/0.3
2	2/0	2/1	2/1	2/0	1/0	0/0	2.0/0.7
3	2/0	2/0	2/0	2/0	3/1	2/1	2.0/0.0
4	2/0	2/0	2/0	1/0	1/0	1/0	1.7/0.0
5	2/0	2/1	2/1	2/1	2/1	0/0	2.0/1.0
6	1/0	2/1	2/0	2/0	2/0	0/0	2.0/0.3

According to the 2nd criterion for classification for skin irritation (category 2), when inflammation persists to the end of the observation period in 2 or more test animals, taking into consideration alopecia (limited area), hyperkeratosis, hyperplasia and scaling, then a test material shall be considered to be an irritant. RAC considers that this criterion is met for pyraclostrobin as inflammation observed in two animals of the OECD TG 404 study persisted to the end of the observation period.

In addition to the skin irritation noted in the GPMT (OECD TG 406), erythema was also observed in all animals in the acute dermal toxicity study (OECD TG 402) and dose-related signs of local irritation were observed at all dose levels in the area of the treated skin in the 28-d repeated dose dermal toxicity study in Wistar rats with pyraclostrobin (OECD TG 410). These studies provide supporting information on the skin irritation potential of pyraclostrobin.

Overall, RAC agrees with the DS and concludes that **pyraclostrobin warrants classification as Skin Irrit. 2; H315.**

10.3 Serious eye damage/eye irritation

Table 32: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference

Acute eye irritation OECD 405 GLP-compliant No deviation	Rabbit (NZW) 1 M and 5 F	Pyraclostrobin (purity 98.2 %)	33 mg (in 0.1 mL) 24 h Up to 8-d observation period	Not irritant - Only minor conjunctival effects (redness and swelling) observed during 24-72 h after application - Mean score for redness (24-72 h): 1.7/6 animals - Mean score for swelling (24-72 h): 0.6/6 animals - Fully reversible by day 8 after application	TOX2000-713: 1998

Table 33: Summary table of human data on serious eye damage/eye irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
N/A				

Table 34: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
N/A				

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

In the acute eye irritation study performed in accordance with OECD Guideline 405, pyraclostrobin (~33 mg in 0.1 mL) was applied onto the conjunctival sac of New Zealand white rabbits. No mortality was observed. The rabbits showed some minor conjunctival effects (i.e. redness and/or swelling) observed around 24-72 h after application but they were fully reversible by day 8 after application.

10.3.2 Comparison with the CLP criteria

Toxicological result (pyraclostrobin)	CLP criteria
<u>OECD 405 rabbit study</u> Mean scores (24-72 h): corneal opacity: no animal ≥1 iris lesion: no animal ≥1 conjunctival redness: 3/6 animals ≥2 conjunctival oedema (chemosis): no animal ≥2	Irritating to eyes (Category 2, H319): at least in 2/3 tested animals with a positive response of: corneal opacity: ≥1 and/or iritis: ≥1 and/or conjunctival redness: ≥2 and/or conjunctival oedema (chemosis): ≥2

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

No classification for serious eye damage/eye irritation is warranted.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

Based on an acute eye irritation/corrosion study (OECD TG 405) with pyraclostrobin, the DS proposed no classification for eye irritation.

Comments received during consultation

Two MSCAs commented in support of the DS proposal.

Assessment and comparison with the classification criteria

In the acute eye irritation/corrosion study (OECD TG 405, GLP-compliant and no deviations), 33 mg of pyraclostrobin (purity: 98.2%) was applied to the conjunctival sac of the right eyelid of one male and five female New Zealand White rabbits (observation period: 8 days post application). Only conjunctival effects (redness and swelling) were observed that were fully reversible by 8 days (TOX2000-713: 1998). See table below for mean scores (after grading at 24, 48 and 72 hours).

***Table**: Eye irritation mean scores (of 24, 48 and 72 h values) from the OECD TG 405 study (TOX2000-713: 1998) (Table B.6.2-7 in RAR Vol. 3CA - B.6)*

Animal Number	Opacity	Iris	Conjunctiva		Additional signs
			Redness	Swelling	
1	0.0	0.0	1.3	1.0	Loss of hair at margins of eyelids in all animals.
2	0.0	0.0	2.0	1.0	
3	0.0	0.0	2.0	0.3	
4	0.0	0.0	2.0	0.3	
5	0.0	0.0	1.3	0.7	
6	0.0	0.0	1.3	0.3	

According to the CLP guidance (2017), section 3.3.2.3.2.2 (pg. 310), conjunctival redness and/or swelling mean scores of ≥ 2 in at least 4 out of 6 rabbits would lead to classification for eye irritation in Category 2. In the OECD TG 405 study with pyraclostrobin, only 3 out of 6 rabbits had a mean conjunctival redness score of 2 and the rest of the conjunctival redness scores and all the conjunctival swelling scores were < 2. Thus, RAC agrees with the DS and concludes that **pyraclostrobin warrants no classification for serious eye damage/eye irritation.**

10.4 Respiratory sensitisation

Table 35: Summary table of animal studies on respiratory sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
N/A					

Table 36: Summary table of human data on respiratory sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
N/A				

Table 37: Summary table of other studies relevant for respiratory sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
N/A				

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

No human or animal data on respiratory sensitisation potential of pyraclostrobin were available for evaluation.

10.4.2 Comparison with the CLP criteria

Comparison with the CLP criteria was not possible due to the lack of human data on respiratory sensitising potential of pyraclostrobin. Nevertheless, data from the GPMT test indicate that pyraclostrobin is not sensitising to the skin.

10.4.3 Conclusion on classification and labelling for respiratory sensitisation

Data are lacking to indicate respiratory sensitisation potential of pyraclostrobin, so no classification is warranted.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The DS stated in the CLH report that no human or animal data on respiratory sensitisation potential of pyraclostrobin were available for evaluation and thus proposed no classification.

Comments received during consultation

One Company/manufacturer) commented in support of the DS proposal.

Assessment and comparison with the classification criteria

RAC agrees with the DS that **pyraclostrobin warrants no classification for respiratory sensitisation due to lack of data.**

10.5 Skin sensitisation

Table 38: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
Skin sensitisation OECD 406 GLP-compliant No deviations	Guinea pig (Dunkin-Hartley), F 20/test group 10/control	Pyraclostrobin (purity 99.0%)	Induction (intradermal or percutaneous): 5% Challenge: 1% (maximum non-irritant concentration) Two challenges performed 14 and 21 days after percutaneous induction; patch application for 24 h Reading at 24 and/or 48 h after patch removal	Not sensitising	TOX2000-714: 1998

Table 39: Summary table of human data on skin sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
N/A				

Table 40: Summary table of other studies relevant for skin sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
N/A				

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

The skin sensitising potential of pyraclostrobin was examined in the guinea pig maximisation test (based on the method of Magnusson and Kligman) performed in accordance with OECD Guideline 406. Following both intradermal and percutaneous inductions, skin irritation was observed in all treated animals. Treatment with vehicle alone caused no dermal irritation. No skin findings occurred after the first and second challenges.

10.5.2 Comparison with the CLP criteria

Table 41: Comparison with the CLP criteria

Toxicological result (pyraclostrobin)	**CLP criteria**
0% animals positive for sensitisation after 2 challenges (1% concentration) with intradermal or percutaneous induction (5% concentration)	Classification based on guinea pig maximisation test Category 1A (H317): * ≥30% responding at ≤0.1% intradermal induction dose or * ≥60% responding at >0.1% to ≤1% intradermal induction dose Category 1B (H317): * ≥30% to <60% responding at >0.1% to ≤1% intradermal induction dose or ≥30% responding at >1% intradermal induction dose

10.5.3 Conclusion on classification and labelling for skin sensitisation

No classification for skin sensitisation is warranted.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Based on no skin sensitisation effects in the GPMT (OECD TG 406) with pyraclostrobin, the DS proposed no classification.

Comments received during consultation

Two MSCAs commented in support of the DS proposal.

Assessment and comparison with the classification criteria

In the skin sensitisation study (GPMT; Magnusson and Kligman method; OECD TG 406, GLP-compliant, no deviations), 20 animals were given intradermal and percutaneous induction doses of 5% and a topical challenge dose of 1% (maximum non-irritant concentration, on days 14 and 21) of pyraclostrobin (purity: 99%, vehicle: 1% Tylose CB 30,000 in aqua bidest). No skin findings were observed after the 1st and 2nd challenges (TOX2000-714: 1998). Thus, RAC agrees with the DS and concludes that **pyraclostrobin warrants no classification for skin sensitisation.**

10.6 Germ cell mutagenicity

Table 42: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
Bacterial reverse mutation assay OECD 471 GLP-compliant No deviation	Pyraclostrobin (purity 98.2%)	*S. typhimurium* TA1535, TA100, TA1537, TA98, *E. coli* WP2 uvrA Concentrations up to 5000 µg/plate; without and with S-9 mix	Negative	TOX2000-720: Engelhardt and Hoffmann, 1997
Bacterial reverse mutation assay OECD 471 GLP-compliant; No deviation	Pyraclostrobin (purity 99.2%)	*S. typhimurium* TA1535, TA100, TA1537, TA98, *E. coli* WP2 uvrA Concentrations up to 5000 µg/plate; without and with S-9 mix	Negative	TOX2003-1219: Engelhardt and Leibold, 2002
HPRT locus mammalian cell mutagenicity test OECD 476 GLP-compliant No deviation	Pyraclostrobin (purity 98.2%)	Chinese Hamster ovary (CHO) cells Concentrations up to 20 µg/ml; without and with S-9 mix	Negative	TOX2000-721: Engelhardt and Hoffmann, 1998
Unscheduled DNA synthesis in primary rat hepatocytes OECD 482 GLP-compliant No deviation	Pyraclostrobin (purity 98.2%)	Dose range: 0-1.0 µg/mL	Negative	TOX2000-723: Engelhardt and Hoffmann, 1998
Chromosome aberration assay in mammalian cells OECD 473 GLP-compliant No deviation	Pyraclostrobin (purity 98.2%)	Chinese hamster V79 cells Wide range of concentrations up to 25 µg/mL; without and with S-9 mix	Negative	TOX2000-722: Engelhardt and Hoffmann, 1999
Micronucleus test in human lymphocytes OECD 487 (2016) GLP-compliant Deviation: A total of 1000 binucleate cells per concentration or control were scored instead of 2000 cells as recommended in the guideline.	Pyraclostrobin (purity 99.02%)	Human lymphocytes extracted from one donor (31-years-old healthy, non-smoking and non-medicated female) Dose range: 0.228-12.8 µg/mL with 2 exposure periods (4 or 20 h); without and with S-9 mix	Negative	ASB2019-10762: Naumann, 2018
Cytokinesis-block micronucleus assay in human lymphocytes (G0 phase and proliferating) Peer-reviewed publication No mention of GLP or OECD guideline Supplementary data	Pyraclostrobin (purity 99.9%)	Peripheral lymphocytes extracted from 2 healthy donors (aged 25-28; one male/one female; non-smoking and non-medicated) Up to 6.0 µg/mL (G0 phase), up to 0.75 µg/mL (proliferating lymphocytes); no testing with metabolic activation	Positive (proliferating lymphocytes) Equivocal (G0 phase cells)	ASB2015-11605: Çayir et al., 2012

Table 43: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Chromosome aberration (bone marrow micronucleus assay) OECD 474 NMRI mice, M/F 5/sex/group GLP-compliant No deviation	Pyraclostrobin (purity 98.2%)	0, 75, 150 and 300 mg/kg bw Single oral gavage administration Sampling after 24 or 48 hours Bone marrow exposure demonstrated via radioactivity detection in a separate toxicokinetic study in NMRI mice exposed to a single oral dose of 300 mg/kg bw of radiolabelled pyraclostrobin	Negative	TOX2000-724: 1998

Table 44: Summary table of human data relevant for germ cell mutagenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
N/A				

10.6.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

The genotoxic potential of pyraclostrobin in bacteria, mammalian cells and *in vivo* has been examined in a number of OECD Guideline tests, which unequivocally showed negative outcomes. One scientific peer-reviewed publication (ASB2015-11605: Çayir et al., 2012) reported positive genotoxic outcomes (e.g. micronucleus formation in human lymphocytes) with pyraclostrobin, but this study has significant deviations (e.g. in exposure scheme of the different substances and data analysis) from the corresponding OECD Guideline 487. Furthermore, a micronucleus test in human lymphocytes with pyraclostrobin (ASB2019-10762: Naumann, 2018) was performed in accordance with OECD Guideline 487, which showed negative outcomes.

10.6.2 Comparison with the CLP criteria

With regard to classification and labelling, a comparison with the criteria in the CLP regulation was made. The following criteria for classification for germ cell mutagens are given in the CLP regulation:

Table 45: CLP criteria for germ cell mutagenicity

CLP criteria
The classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans. The classification in Category 1B is based on: — positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or — positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells *in vivo*, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or — positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people. The classification in Category 2 is based on: — positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from: — somatic cell mutagenicity tests *in vivo*, in mammals; or

CLP criteria
— other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays. Note: Substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

No human data are available for pyraclostrobin; hence, a classification in category 1A is not possible.

Neither *in vivo* heritable germ cell mutagenicity tests nor positive results from *in vivo* somatic cell mutagenicity tests in mammals are available or demonstrated; hence, a classification in 1B is also not possible. *In vitro* somatic cell mutagenicity studies performed in accordance with OECD Guidelines were all negative.

The only positive *in vitro* finding was observed in a micronucleus test using human lymphocytes as reported in the scientific publication ASB2015-11605: Çayir et al., 2012 albeit with significant deviations from the corresponding OECD Guideline 487 study. Subsequently, an OECD Guideline 487 study (micronucleus test in human lymphocytes) was conducted (ASB2019-10762: Naumann, 2018) and contravened the positive findings in the ASB2015-11605: Çayir et al., 2012 study. Therefore, the criteria for classification in Category 2 were considered not met based on the submitted data.

10.6.3 Conclusion on classification and labelling for germ cell mutagenicity

No classification for germ cell mutagenicity is warranted.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Based on the negative results from several standard *in vitro* and an *in vivo* mutagenicity/genotoxicity studies with pyraclostrobin, the DS proposed no classification for germ cell mutagenicity.

Comments received during consultation

Two MSCAs and a Company/manufacturer supported the DS proposal. The Company/manufacturer also commented that the *in vitro* micronucleus test in human lymphocytes (ASB2019-10762: 2018) was a range-finding study which explains the guideline (OECD TG 487) deviation of scoring only 1000 cells instead of 2000 (see table further below). The company further noted that the results were negative when it tested pyraclostrobin batches (spiked with low amounts of technical impurities) in 9 *in vitro* micronucleus tests in human lymphocytes (guideline- and GLP-compliant). These studies are available in the confidential volume 4 of the RAR.

Assessment and comparison with the classification criteria

***Table**: Summary table of mutagenicity/genotoxicity tests with pyraclostrobin (Tables 42 and 43 in the CLH report)*

Method, test guideline, deviations if any	Test substance	Relevant information about the study	Observations	Reference
In vitro				
Bacterial reverse mutation assay OECD TG 471 GLP-compliant No deviation	Pyraclostrobin (purity 98.2%)	*S. typhimurium* TA1535, TA100, TA1537, TA98, *E. coli* WP2 uvrA Concentrations up to 5000 µg/plate; without and with S-9 mix	Negative	TOX2000-720: 1997
Bacterial reverse mutation assay OECD TG 471 GLP-compliant; No deviation	Pyraclostrobin (purity 99.2%)	*S. typhimurium* TA1535, TA100, TA1537, TA98, *E. coli* WP2 uvrA Concentrations up to 5000 µg/plate; without and with S-9 mix	Negative	TOX2003-1219: 2002
HPRT locus mammalian cell mutagenicity test OECD TG 476 GLP-compliant No deviation	Pyraclostrobin (purity 98.2%)	Chinese Hamster ovary (CHO) cells Concentrations up to 20 µg/ml; without and with S-9 mix	Negative	TOX2000-721: 1998
Unscheduled DNA synthesis in primary rat hepatocytes OECD TG 482 GLP-compliant No deviation	Pyraclostrobin (purity 98.2%)	Dose range: 0-1.0 µg/mL	Negative	TOX2000-723: 1998
Chromosome aberration assay in mammalian cells OECD TG 473 GLP-compliant No deviation	Pyraclostrobin (purity 98.2%)	Chinese hamster V79 cells Wide range of concentrations up to 25 µg/mL; without and with S-9 mix	Negative	TOX2000-722: 1999
Micronucleus test in human lymphocytes OECD TG 487 (2016) GLP-compliant Deviation: A total of 1000 binucleate cells per concentration or control were scored instead of 2000 cells as recommended in the guideline.	Pyraclostrobin (purity 99.02%)	Human lymphocytes extracted from one donor (31-years-old healthy, non-smoking and non-medicated female) Dose range: 0.228-12.8 µg/mL with 2 exposure periods (4 or 20 h); without and with S-9 mix	Negative	ASB2019-10762: 2018
Cytokinesis-block micronucleus assay in human lymphocytes (G0 phase and proliferating) Peer-reviewed publication No mention of GLP or OECD TG Supplementary data	Pyraclostrobin (purity 99.9%)	Peripheral lymphocytes extracted from 2 healthy donors (aged 25-28; one male/one female; non-smoking and non-medicated) Up to 6.0 µg/mL (G0 phase), up to 0.75 µg/mL (proliferating lymphocytes); no testing with metabolic activation	Positive (proliferating lymphocytes) Equivocal (G0 phase cells)	ASB2015-11605: 2012

In vivo				
Chromosome aberration (bone marrow micronucleus assay) OECD TG 474 NMRI mice, M/F 5/sex/group GLP-compliant No deviation	Pyraclostrobin (purity 98.2%)	0, 75, 150 and 300 mg/kg bw Single oral gavage administration Sampling after 24 or 48 hours Bone marrow exposure demonstrated via radioactivity detection in a separate toxicokinetic study in NMRI mice exposed to a single oral dose of 300 mg/kg bw of radiolabelled pyraclostrobin	Negative	TOX2000-724: 1998

No human data relevant for germ cell mutagenicity is available for pyraclostrobin.

The standard *in vitro* assays (Ames, gene mutation, chromosome aberration and UDS tests) were negative. In a publication (ASB2015-11605: 2012), pyraclostrobin was found to be positive in a micronucleus test in human lymphocytes. However, this publication is considered only supplemental by the DS as it had 'significant deviations' from the corresponding test guideline OECD TG 487. In any case, negative results from the standard *in vitro* (OECD TG 487, GLP-compliant; ASB2019-10762: 2018) and *in vivo* micronucleus tests (OECD TG 474, GLP-compliant; TOX2000-724: 1998) removes concern for chromosome aberrations.

Therefore, RAC agrees with the DS and concludes that **pyraclostrobin warrants no classification for germ cell mutagenicity.**

10.7 Carcinogenicity

Table 46: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Carcinogenicity study OECD 451 Wistar rats, M/F 50/sex/group GLP-compliant No deviation	Pyraclostrobin (purity 97.09 %) 0, 25, 75, 200 ppm <u>Test substance intake</u> M: 0, 1.2, 3.4, 9.2 mg/kg bw/day F: 0, 1.5, 4.7, 12.6 mg/kg bw/day 24-month oral dietary exposure	No indication of carcinogenic potential	TOX2000-727: 1999
Carcinogenicity study OECD 451	Pyraclostrobin (purity 97.09 %)	No indication of carcinogenic potential	TOX2000-728: 1999

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
B6C3F1 mice, M/F 50/sex/group GLP-compliant No deviation	0, 10, 30, 120 ppm (M/F), 180 ppm (F only) Test substance intake M: 0, 1.4, 4.1, 17.2 mg/kg bw/day F: 0, 1.6, 4.8, 20.5, 32.8 mg/kg bw/day 18-month oral dietary exposure		

Table 47: Summary table of human data on carcinogenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
N/A				

Table 48: Summary table of other studies relevant for carcinogenicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Chronic toxicity OECD 452 Rat (Wistar) M/F 20/sex/group GLP-compliant No deviations	Pyraclostrobin (purity 97.09%)	Oral administration via the diet of 0, 25, 75 and 200 ppm (calculated daily substance intake of 0, 1.1, 3.4 and 9.0 mg/kg bw/d for males and 0, 1.5, 4.6 and 12.3 mg/kg bw/d for females) pyraclostrobin for 24 months	Leydig cell tumour (LCT) was commonly observed in a number of males of all groups including control. No dose-response relationship was observed (see Table below).	TOX2000-726: 1999

Dose	0	25	75	200
# males	20	20	20	20
# LCT	9	12	11	8
%	45	60	55	40

10.7.1 Short summary and overall relevance of the provided information on carcinogenicity

Table 49: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
N/A								

10.7.2 Comparison with the CLP criteria

The following criteria (briefly described here) for classification for carcinogenicity are given in the CLP Regulation:

Table 50: Comparison with the CLP criteria for carcinogenicity

CLP criteria
A substance is classified in Category 1 (known or presumed human carcinogens) for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as: Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence. The classification in Category 1A and 1B is based on strength of evidence together with additional considerations. Such evidence may be derived from: - human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or - animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen). The placing of a substance in Category 2 (suspected human carcinogens) is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited (1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

For pyraclostrobin, there are no relevant data from epidemiological studies; hence, no classification with Category 1A according to CLP regulation can be proposed.

Long-term/carcinogenicity dietary toxicity studies were conducted in rats and mice according to relevant OECD test guidelines (451) and GLP principles. In both studies, no increased incidences of tumours were reported under the conditions of the studies. The only tumour finding was Leydig cell tumours found in males, but this tumour type was considered a common tumour finding in this animal model. No dose-response relationship was observed, and the incidence rate was within the range of the laboratory's historical control data for this tumour type.

10.7.3 Conclusion on classification and labelling for carcinogenicity

No classification for carcinogenicity is warranted.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification for carcinogenicity as there were no indications in the standard carcinogenicity studies (OECD TG 451, GLP-compliant and no deviations) in Wistar rats and B6C3F1 mice. In a chronic toxicity study (OECD TG 452, GLP-compliant and no deviations) in Wistar rats, Leydig cell tumours (which are common for this strain) were observed but without a dose response and within the range of historical control data (HCD).

Comments received during consultation

Three MSCAs supported the DS proposal. One of these requested more information on the HCD for Leydig cell tumours. The information provided by the DS in response is reflected by RAC further below in its assessment.

Assessment and comparison with the classification criteria

***Table**: Summary table of the carcinogenicity studies and the chronic study with pyraclostrobin (adapted from Tables 46 and 48 in the CLH report by adding general toxicity observations)*

Method, guideline, deviations if any	Test substance, dose levels duration of exposure	Observations	Reference
Carcinogenicity study OECD TG 451 Wistar rats, M/F 50/sex/group GLP-compliant No deviation	Pyraclostrobin (purity 97.09 %) 0, 25, 75, 200 ppm Test substance intake M: 0, 1.2, 3.4, 9.2 mg/kg bw/d F: 0, 1.5, 4.7, 12.6 mg/kg bw/d 24-month oral dietary exposure	No indication of carcinogenic potential. There was no test substance related increase in mortality or clinical signs of toxicity. Survival rate and other details are lacking. Reduced BW (-4% M; -13.7% F) and BWG (-4.9% M; -21.7% F) were observed in the high dose at the end of the study.	TOX2000-727: 1999
Carcinogenicity study OECD TG 451 B6C3F1 mice, M/F 50/sex/group GLP-compliant No deviation	Pyraclostrobin (purity 97.09 %) 0, 10, 30, 120 ppm (M/F), 180 ppm (F only) Test substance intake M: 0, 1.4, 4.1, 17.2 mg/kg bw/d F: 0, 1.6, 4.8, 20.5, 32.8 mg/kg bw/d 18-month oral dietary exposure	No indication of carcinogenic potential. There was no test substance related increase in mortality and no clinical signs of toxicity were observed. Survival rate and other details are lacking. Reduced BW (-13% M; -9,5% F) and BWG (-28% M; -20%F) were observed at 17.2/20.5 mg/kg bw/d.	TOX2000-728: 1999
Chronic toxicity OECD TG 452 Rat (Wistar) M/F 20/sex/group GLP-compliant No deviations	Pyraclostrobin (purity 97.09%) Oral administration via the diet of 0, 25, 75 and 200 ppm (calculated daily substance intake of 0, 1.1, 3.4 and 9.0 mg/kg bw/d for males and 0, 1.5, 4.6 and 12.3 mg/kg bw/d for females) pyraclostrobin for 24 months	Leydig cell tumour (LCT) was commonly observed in a number of males of all groups including control. No dose-response relationship was observed (see Table below). **Dose / 0 / 25 / 75 / 200** # males: 20 / 20 / 20 / 20 # LCT: 9 / 12 / 11 / 8 %: 45 / 60 / 55 / 40 There were no test substance-related increases in mortality or clinical signs of toxicity. There were no effects on food consumption and body weight (no stat. sig. changes).	TOX2000-726: 1999

No human data relevant to evaluate carcinogenic potential is available for pyraclostrobin.

In the available animal studies, the only indication of carcinogenic potential was observed in the chronic toxicity study in Wistar rats in which there was a dose independent increase in Leydig cell tumours in the low- (60% of males) and mid dose (55%) groups compared to controls (45%). The incidences were lower in the high dose group (40%). The laboratory's HCD showed a range of 30 – 60% for Leydig cell tumours in the 24 chronic toxicity studies performed in Wistar rats between 1990 and 2000. Both the minimum and maximum incidence rates were observed between 1996 and 1997 which is within the 5-year period of the study.

There were no increased incidences of tumours in the standard carcinogenicity studies in Wistar rats and B6C3F1 mice. Therefore, RAC agrees with the DS and concludes that **pyraclostrobin warrants no classification for carcinogenicity.**

10.8 Reproductive toxicity

10.8.1 Adverse effects on sexual function and fertility

Table 51: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Two-generation reproduction toxicity study, OECD 416 Wistar rats; M/F 25/sex/group GLP-compliant Deviation: From day 14 after parturition onwards, food consumption was not determined since the pups themselves began to consume considerable amounts of solid food. This deviation did not alter the validity of the results.	Pyraclostrobin (purity 98.7 %) 0, 25, 75, 300 ppm Daily test substance intake 0, 2.7, 8.2, 32.6 mg/kg bw/day (calculated for the whole study period with all phases, for all groups and both sexes) Dietary exposure F_0: Treatment started 74 days before mating and lasted until after weaning of F_1 pups. F_1: Exposed continuously from their growth into adulthood until or up to 16 hours before sacrifice (i.e. after weaning of F_2 pups).	NOAELs: Parental: ~8.2 mg/kg bw/day [75 ppm] Reproductive: ~32.6 mg/kg bw/day [300 ppm] Offspring: ~8.2 mg/kg bw/day [75 ppm] Reported effects Parental toxicity: Reduced food consumption (by around 5 %) in high dose males and females, slightly decreased body weight/gain in top dose males and females (mostly ≤ 5 % and not significant); relative kidney weight in F_0 and F_1 males significantly increased by 5.6 or 7.0 %, respectively Reproductive toxicity: No treatment-related effects observed Offspring: Significantly reduced pup body weight gain (F_1 and F_2) in high dose groups (by ca 10 %), delay in vaginal opening in F_1 high dose female pups by about 1.6 days as compared to the	TOX2000-729: 1999

		control, might be related to lower bw	
One-generation reproduction toxicity, dose range-finding study Similar to OECD 415 Wistar rats; M/F 10/sex/group GLP-compliant Deviations: - 10 pregnant females examined instead of 20 - F0 generation parental animals were mated from 45 days onwards after the beginning of treatment, whereas it is required in the guideline that dosing is continued for ten weeks prior to the mating period - food consumption of the females during the mating period was not determined Supplementary data	Pyraclostrobin (purity 97.09 %) 0, 200, 400, 600 ppm <u>Test substance intake</u> M: 0, 20.5, 39.9, 59.1 mg/kg bw/day F (three phases) - premating: 0, 21.3, 42.5, 60.4 mg/kg bw/day - gestation: 0, 18.3, 35.0, 53.2 mg/kg bw/day - lactation (PND 1-14): 0, 29.0, 51.9, 80.2 mg/kg bw/day Dietary exposure Treatment started 45 days before mating and lasted until day 21 after parturition (weaning of F_1 pups).	<u>NOAEL</u>: not established as this was a dose range-finding study <u>Reported effects</u> <u>Parental toxicity</u>: Statistically significant and dose-related reductions in food consumption (up to 10 % in males and 15 % in females at some stages of the study) and significant decreases in body weight/bw gain (up to 10 – 20 % in males and 6 – 18 % in females) at the two upper dose levels; Anaemia in mid and high dose males and females; Organ weight changes in parental males: significant decreases of absolute liver (up to 15 % reduction) and kidney (by ca 13 %) weights as well as the statistically significant increases of relative testes (by up to 21 %) and epididymis weights (+22 %) in mid and high dose groups not accompanied by respective changes of relative (liver & kidney) or absolute (testes & epididymis) weights or by any gross lesions in these organs. The only gross pathology finding was a thickening of the wall of the duodenum in all males at 600 ppm <u>Reproductive toxicity</u>: No effects on fertility and reproductive performance were observed up to the highest dose tested. <u>Developmental toxicity</u>: Impaired body weight development of pups at all dose levels.	ASB2017-5538: 2002

Table 52: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
N/A				

Table 53: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Chronic toxicity	Pyraclostrobin (purity 97.09%)	Oral administration via the diet of 0, 25, 75 and 200	In the testes, unilateral or bilateral calcification (incidence of 3/0/4/4,	TOX2000-726: 1999

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
OECD 452 Rat (Wistar) M/F 20/sex/group GLP-compliant No deviations		ppm (calculated daily substance intake of 0, 1.1, 3.4 and 9.0 mg/kg bw/d for males and 0, 1.5, 4.6 and 12.3 mg/kg bw/d for females) pyraclostrobin for 24 months	corresponding to 0, 25, 75 and 200 ppm, respectively) and cystic degeneration (3/5/6/7) were slightly more often noted in treated males than in control males. However, focal lesions (6/5/8/2) of various size, number and/or colour were noted with a higher incidence in control than in the high dose group. There were higher but not dose-related incidences of tubular degeneration (1/7/7/6) and tubular mineralisation (1/4/6/4) in the testes of all treated groups. However, these findings were often associated with tubular atrophy (9/5/5/6) and Leydig cell tumours (9/12/11/8), and they are, hence, not regarded as treatment-related findings but being secondary events to atrophy and/or Leydig cell tumours that are commonly observed in this animal model (refer to Section 10.9).	

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

A 2-generation reproduction toxicity study (accompanied by a preceding 1-generation dose range-finding study) in Wistar rats performed in accordance with OECD Guideline 416 was available for the evaluation of the potential effects of pyraclostrobin on sexual function and fertility. Both the dose range-finding 1-generation reproduction study and the main 2-generation reproduction study did not observe any effects of pyraclostrobin on fertility or reproductive performance up to the highest tested dose. Testicular effects observed in the 2-year chronic toxicity study in rats were attributable to the animal model and were not considered as treatment-related.

10.8.3 Comparison with the CLP criteria

Please refer to Section 10.10.9 for the comparison.

10.8.4 Adverse effects on development

Table 54: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Prenatal developmental toxicity OECD 414 Wistar rats; F (pregnant) 25/group GLP-compliant	Pyraclostrobin (purity 98.9 %) 0, 10, 25, 50 mg/kg bw/day Daily oral gavage gestation days 6-19	NOAELs: Maternal: 10 mg/kg bw/day Developmental: 25 mg/kg bw/day Reported effects Maternal toxicity: Significantly reduced food consumption on the	TOX2000-730: 1999

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
No deviations		first days of treatment (days 6 – 8) with decreases by 14 % in the mid dose group and by 27 % in high dose dams resulting in a significantly lower corrected body weight gain in both groups (-22 % and -45 %, respectively) Developmental toxicity: No treatment-related malformations; Increase in soft tissue and skeletal variations observed at highest dose (see below)	
Prenatal developmental toxicity OECD 414 Himalayan rabbits; F (pregnant) 25/group GLP-compliant No deviations	Pyraclostrobin (purity 98.9%) 0, 5, 10, 20 mg/kg bw/day Daily oral dose gestation days 7-28	NOAELs: Maternal: <5 mg/kg bw/day Developmental: 5 mg/kg bw/day Reported effects Maternal toxicity: Significantly lower food intake (by 64, 79, and even 89 % at the low, mid, and high dose level) on the first two days of dosing, resulting in severe initial body weight losses in all dose groups and a significanly reduced total bw gain over the study period at the two upper dose levels (-39 % in the mid dose group and -77 % in the top dose group) Developmental toxicity: Increased post-implantation losses and early resorptions (e.g. complete embryolethality) at two upper dose levels; increase in total frequency of skeletal malformations and in particular of absent lumbar vertebrae at 20 mg/kg bw/d (see below)	TOX2000-731: 1999
Two-generation reproduction toxicity study, OECD 416 Wistar rats; M/F 25/sex/group GLP-compliant Deviation: From day 14 after parturition onwards, food consumption was not determined since the pups themselves began to consume considerable amounts of solid food. This deviation did not alter the validity of the results.	Pyraclostrobin (purity 98.7 %) 0, 25, 75, 300 ppm Daily test substance intake 0, 2.7, 8.2, 32.6 mg/kg bw/day (calculated for the whole study period with all phases, for all groups and both sexes) Dietary exposure F_0: Treatment started 74 days before mating and	NOAELs Parental and offspring: ~8.2 mg/kg bw/day [75 ppm] Reported effects Parental: Reduced food consumption, body weight and body weight gain in male and female parental animals in both generations (for details, see Table 51: Summary table of animal studies on adverse effects on sexual function and fertility)	TOX2000-729: 1999

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
	lasted until after weaning of F_1 pups. F_1: Exposed continuously from their growth into adulthood until or up to 16 hours before sacrifice (i.e. after weaning of F_2 pups).	Offspring: Reduced pup body weight gain (F_1 and F_2), delay in vaginal opening (F_1 only)	
One-generation reproduction toxicity, dose range-finding study Similar to OECD 415 Wistar rats; M/F 10/sex/group GLP-compliant Deviations: - 10 pregnant females examined instead of 20 - F0 generation parental animals were mated from 45 days onwards after the beginning of treatment, whereas it is required in the guideline that dosing is continued for ten weeks prior to the mating period - food consumption of the females during the mating period was not determined Supplementary data	Pyraclostrobin (purity 97.09 %) 0, 200, 400, 600 ppm Test substance intake M: 0, 20.5, 39.9, 59.1 mg/kg bw/day F (three phases) - premating: 0, 21.3, 42.5, 60.4 mg/kg bw/day - gestation: 0, 18.3, 35.0, 53.2 mg/kg bw/day - lactation (PND 1-14): 0, 29.0, 51.9, 80.2 mg/kg bw/day Dietary exposure Treatment started 45 days before mating and lasted until day 21 after parturition (weaning of F_1 pups).	Indications of developmental toxicity consisted of impaired body weight development of pups at all dose levels.	ASB2017-5538: 2002
Maternal toxicity Similar to OECD 414 Himalayan rabbits; F (pregnant) 25/group GLP-compliant Deviation: With regard to examination for foetal effects, the range of parameters investigated was limited since the focus of this additional study was on maternal toxicity. Foetuses were only removed, counted and weighed. Supplementary data	Pyraclostrobin (purity 98.9 %) 0, 1, 3 and 5 mg/kg bw/d Daily oral dose Days 7-28 post-insemination	NOAELs: Maternal: 3 mg/kg bw/day Developmental: Not established, not investigated Reported effects Maternal toxicity: Statistically significant decreases in initial food consumption (days 7 and 8) in mid and high dose does (-26 % and –41 %, respectively) and initial slight body weight loss at the high dose level Developmental toxicity: Not investigated in full but no evidence of gross malformations	TOX2001-471: 2001

In the following additional tables, the impact of test substance administration on maternal body weight and food consumption in the individual developmental studies and also on pup weight in the two-generation study (TOX2000-729: 1999) is given in more detail in order to further substantiate the summary results in Table 54 above.

Table 55: Two-generation study in rats (TOX2000-729: 1999): Body weight data of F0 parental animals during different study phases

	Time period	Control	25 ppm		75 ppm		300 ppm	
		Mean ± SD	Mean ± SD	%	Mean ± SD	%	Mean ± SD	%
BW males premating	Week 0	129.5 ± 14.5	128.8 ± 13.6	-0.5	128.8 ± 14.5	-0.5	128.7 ± 14.4	-0.6
	Week 10	416.8 ± 27.3	421.1 ± 35.7	1.0	424.9 ± 24.7	1.9	402.9 ± 31.0	-3.3
BWG males premating[#]	Week 1-10	287.3	292.3	1.7	296.1	3.1	274.2	-4.6
BW males postmating	Week 21	487.3 ± 31.2	505.1 ± 48.7	3.7	501.4 ± 37.9	2.9	474.1 ± 38.1	-2.7
BWG males	Week 0-21	357.7 ± 29.0	376.3 ± 47.8	5.2	372.6 ± 34.3	4.2	345.3 ± 35.0	-3.5
BW females premating	Week 0	118.5 ± 5.9	118.8 ± 8.6	0.3	117.9 ± 6.1	-0.5	118.2 ± 6.4	-0.3
	Week 10	263.1 ± 12.5	263.9 ± 21.5	0.3	265.0 ± 19.9	0.7	252.3 ± 14.2	-4.1
BWG females premating	Week 0-10	144.6 ± 11.8	145.0 ± 19.8	0.3	147.1 ± 16.7	1.7	134.1 ± 11.9	-7.3
BW females postmating	Week 21	303.2 ± 13.5	304.0 ± 27.9	0.3	308.4 ± 22.2	1.7	288.1* ± 18.1	-5.0
BW females gestation	Day 0	266.0 ± 12.1	263.7 ± 23.1	-0.9	266.0 ± 20.4	0.0	252.4* ± 14.6	-5.1
	Day 7	293.5 ± 12.5	290.8 ± 25.2	-0.9	292.5 ± 21.3	-0.3	280.7 ± 15.4	-4.4
	Day 14	324.2 ± 13.2	323.6 ± 26.9	-0.2	323.9 ± 23.5	-0.1	310.9 ± 18.4	-4.1
	Day 20	396.6 ± 22.5	395.8 ± 30.5	-0.2	391.0 ± 28.4	-1.4	378.4* ± 22.6	-4.6
BWG females gestation	Day 0-20	130.6 ± 17.6	132.1 ± 17.5	1.1	125.0 ± 17.3	-4.3	125.9 ± 14.5	-3.6
BW females lactation	Day 1	300.9 ± 17.0	300.6 ± 30.1	-0.1	303.4 ± 24.3	0.8	286.7 ± 18.7	-4.7
	Day 4	315.3 ± 16.6	310.5 ± 29.0	-1.5	314.3 ± 25.1	-0.3	299.8 ± 20.3	-4.9
	Day 7	320.8 ± 16.7	319.3 ± 27.6	-0.5	319.7 ± 23.0	-0.3	311.3 ± 21.6	-3.0
	Day 14	334.5 ± 18.4	334.7 ± 23.5	0.1	333.3 ± 24.3	-0.4	318.1* ± 21.3	-4.9
	Day 21	325.2 ± 17.2	321.3 ± 24.4	-1.2	324.5 ± 21.7	-0.2	310.8 ± 19.5	-4.4
BWG females lactation	Day 0-21	24.3 ± 15.9	20.7 ± 17.7	-14.8	21.0 ± 15.8	-13.6	24.1 ± 13.5	-0.8

BW = Body Weight; BWG = Body Weight Gain; * $p \leq 0.05$, ** $p \leq 0.01$ (Dunnett test, two-sided)

[#] Parameter not calculated in the report, thus no SD is available

Table 56: Two-generation study in rats (TOX2000-729: 1999): Body weight data of F1 parental animals during different study phases

	Time period	Control	25 ppm		75 ppm		300 ppm	
		Mean ± SD	Mean ± SD	%	Mean ± SD	%	Mean ± SD	%
BW males premating	Week 0	85.7 ± 14.9	92.4 ± 9.5	7.8	89.6 ± 10.7	4.6	78.1 ± 9.0	-8.9
	Week 10	412.4 ± 36.4	410.1 ± 26.2	-0.6	439.6* ± 37.5	6.6	388.4* ± 31.9	-5.8
BWG males premating[#]	Week 0-10	326.6	317.6	-2.8	350.1	7.2	310.2	-5.0
BW males postmating	Week 20	499.6 ± 47.7	501.4 ± 38.8	0.4	540.7** ± 47.5	8.2	482.2 ± 36.5	-3.5

	Time period	Control	25 ppm		75 ppm		300 ppm	
		Mean ± SD	Mean ± SD	%	Mean ± SD	%	Mean ± SD	%
BWG males	Week 0-20	413.9 ± 42.7	409.0 ± 38.0	-1.2	451.1** ± 43.1	9.0	404.1 ± 31.7	-2.4
BW females premating	Week 0	80.9 ± 7.5	85.7 ± 10.6	5.9	82.6 ± 9.5	2.1	73.4* ± 7.7	-9.3
	Week 10	250.1 ± 18.2	259.9 ± 17.8	3.9	262.0 ± 17.3	4.8	246.5 ± 17.0	-1.4
BWG females premating	Week 0-10	169.2 ± 17.9	174.2 ± 18.8	3.0	179.5 ± 16.6	6.1	173.1 ± 15.2	2.3
BW females postmating	Week 21	304.8 ± 20.8	309.1 ± 16.5	1.4	309.0 ± 20.2	1.4	289.0* ± 19.5	-5.2
BW females gestation	Day 0	252.9 ± 16.2	262.4 ± 16.4	3.8	263.7 ± 19.4	4.3	248.3 ± 15.4	-1.8
	Day 7	282.1 ± 17.5	289.1 ± 16.8	2.5	291.7 ± 19.8	3.4	278.4 ± 16.7	-1.3
	Day 14	316.5 ± 19.3	322.5 ± 15.5	1.9	322.2 ± 18.4	1.8	308.2 ± 18.0	-2.6
	Day 20	381.5 ± 23.2	384.6 ± 27.4	0.8	378.7 ± 29.0	-0.7	373.6 ± 24.7	-2.1
BWG females gestation	Day 0-20	128.6 ± 18.3	122.2 ± 25.6	-5.0	115.0 ± 29.6	-10.6	125.3 ± 14.9	-2.6
BW females lactation	Day 1	298.6 ± 20.8	302.5 ± 21.8	1.3	294.1 ± 24.6	-1.5	288.3 ± 17.4	-3.4
	Day 4	301.4 ± 20.3	306.7 ± 19.8	1.8	301.9 ± 22.0	0.2	291.3 ± 20.9	-3.4
	Day 7	308.3 ± 22.5	310.6 ± 18.5	0.7	312.5 ± 21.7	1.4	304.1 ± 22.3	-1.4
	Day 14	324.5 ± 22.1	322.7 ± 18.6	-0.6	323.4 ± 24.1	-0.3	314.6 ± 22.1	-3.1
	Day 21	322.4 ± 23.6	322.6 ± 15.8	0.1	321.8 ± 20.5	-0.2	315.2 ± 18.1	-2.2
BWG females lactation	Day 0-21	23.8 ± 12.0	20.1 ± 12.9	-15.5	27.7 ± 15.1	16.4	27.0 ± 13.7	13.4

BW = Body Weight; BWG = Body Weight Gain; * $p \leq 0.05$, ** $p \leq 0.01$ (Dunnett test (two sided))

Parameter not calculated in the report, thus no SD available

Table 57: Two-generation study in rats (TOX2000-729: 1999): Food consumption data of F0 and F1 parental animals during different study phases [g/animal/day]

	Time period	Control	25 ppm		75 ppm		300 ppm	
		Mean ± SD	Mean ± SD	%	Mean ± SD	%	Mean ± SD	%
F0 males premating#	Week 0-10	26.0 ± 1.20	26.5 ± 1.11	1.9	26.6 ± 1.20	2.3	25.3 ± 1.26	-2.7
F0 females premating#	Week 0-10	19.9 ± 0.57	19.9 ± 0.58	0.0	20.0 ± 0.52	0.5	18.8 ± 0.52	-5.5
F0 females gestation#	Day 0-20	25.3 ± 0.84	25.6 ± 0.98	1.2	26.0 ± 0.95	2.8	24.5 ± 1.00	-3.2
F0 females lactation#	Day 1-14	46.3 ± 10.2	45.6 ± 11.1	-1.5	45.0 ± 10.3	-2.8	45.9 ± 9.49	-0.9
F1 males premating#	Week 0-10	26.6 ± 2.94	26.8 ± 2.72	0.8	27.9 ± 3.31	4.9	25.4 ± 3.37	-4.5
F1 females premating#	Week 0-10	20.2 ± 1.44	20.6 ± 1.26	2.0	20.5 ± 1.26	1.5	19.1 ± 1.48	-5.4
F1 females gestation#	Day 0-20	25.9 ± 2.03	25.8 ± 1.78	-0.4	25.7 ± 1.60	-0.8	24.6 ± 1.40	-5.0
F1 females lactation#	Day 1-14	45.2 ± 12.8	45.5 ± 12.2	0.7	42.5 ± 10.3	-6.0	44.5 ± 11.3	-1.5

Reported as mean of the means; no statistics performed

Table 58: Two-generation study in rats (TOX2000-729: 1999): Pup findings

Parameter	Dose level (ppm)			
	0	25	75	300
F1 - Body weight, day 21, m / f (g)	52.8 / 51.4	53.7 / 51.3	52.8 / 50.3	47.4**/ 45.2**
F1 - Vaginal opening (days)	31.7	32.1	32.4	33.3**
F2 - Body weight, day 7, m / f (g)	15.2 / 14.5	14.9 / 14.6	14.7 / 14.2	13.5**/ 13.1**
F2 - Body weight, day 21, m / f (g)	52.0 / 49.8	52.6 / 50.2	51.4 / 48.9	45.0**/ 43.5**

Dunnett's test *p<0.05, **p< 0.01

Table 59: Developmental toxicity study in the rat (TOX2000-730: 1999): Maternal findings

Parameter	Dose level (mg/kg bw/day)			
	0	10	25	50
Food consumption; day 6-8 (g/animal/day)	24.6	23.6	21.2**	17.9**
Food consumption; day 6-19 (g/animal/day)	26.3	26.1	24.5	23.3
Body weight; day 20 (g)	369	373	354	350*
Body weight change; day 6-19 (g)	104	107	96	88**
Gravid uterus (g)	79.4	83.0	77.4	78.0
Carcass weight (g)	290	290	277	272**
Corrected body weight gain (g)	40.7	40.9	31.9*	22.3**

Dunnett's test *p<0.05, **p<0.01

Table 60: Developmental toxicity study in the rabbit (TOX2000-731: 1999): Maternal findings

Parameter	Dose level (mg/kg bw/day)			
	0	5	10	20
Food consumption; day 7-8 (g/animal/day)	98.1	35.7**	20.4**	10.5**
Body weight change; day 7-9 (g)	-3.8	-43.8**	-85.5**	-146.3**
Body weight change; days 7-28 (g)	191.5	131.7	116.0*	44.1**
Body weight; day 29 (g)	2961	2807	2851	2748**
Corrected body weight gain over treatment period (g)	-135.7	-142.4	-132.9	-146.8
Gravid uterus (g)	352.6	302.6	271.2*	209.6**

Dunnett's test *p<0.05, **p<0.01

Table 61: Maternal toxicity study in rabbits (TOX2001-471: 2001): Effects on food intake and body weight

Parameter	Dose level (mg/kg bw/day)			
	0	1	3	5
Food consumption, day 7 – 28 (g/animal/day)	99.0	93.9	84.0	80.0
Food consumption; day 7-8 (g/animal/day)	113.2	107.8	84.2**	66.7**
Body weight; day 29 (g)	2880	2834	2806	2806
Body weight change; day 7-9 (g)	12.8	7.5	-3.8	-14.2**
Gravid uterus (g)	321.8	320.5	306.2	354.4
Corrected body weight gain; from day 7 (g)	-82.9	-102.7	-109.4	-136.8

Dunnett's test *p<0.05, **p<0.01

Table 62: Summary table of human data on adverse effects on development

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
N/A				

Table 63: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
N/A				

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

The developmental toxicity potential of pyraclostrobin was assessed in a number of prenatal developmental toxicity and reproductive toxicity tests performed in accordance with OECD Test Guidelines (e.g. OECD Guideline 414 or 416).

Pyraclostrobin was not shown to be teratogenic in rats although a marginal effect on foetal development cannot be excluded. Reduced pup weight gain (by ca 10 %) was observed in a two-generation study (TOX2000-729: 1999) at the maternally toxic highest dose level of 300 ppm (see Table 58 above) but no increase in malformations was seen. Likewise, there was no significant or dose-related increase in the total frequency of skeletal abnormalities observed in the prenatal developmental toxicity study (TOX2000-730: 1999). With regard to more specific, individual findings, a dose-related increase in incompletelely ossified sternebrae was noted but the difference between the control and the high dose groups was not statistically significant and the incidences were all within the historical control range even though, in the group receiving 50 mg/kg bw per day, at its upper edge. In contrast, the increase in rudimentary cervical ribs in foetues at the high dose level might suggest a weak delay in skeletal maturation in the presence of maternal toxicity (see also Table 59).

Table 64: Developmental toxicity study in the rat (TOX2000-730: 1999): Summary of skeletal variations against the background of historical control data provided by ASB2019-10765: 2019

Findings	Historical control range (min-max, study mean in brackets)[&]	Dose level (mg/kg bw/day)			
		0	10	25	50
Total fetal skeletal variations					
# foetuses affected/evaluated (%)	31 – 95 % (51 %)	129/158 (82 %)	135/151 (89 %)	121/147 (82 %)	162/178 (91 %)
# litters affected/evaluated (%)	80 – 100 % (88.3 %)	22/22 (100 %)	20/20 (100 %)	21/21 (100 %)	25/25 (100 %)
Incomplete ossification of sternebrae					
# foetuses affected/evaluated (%)	14 – 35 % (26.1 %)	29/158 (18 %)	39/151 (26 %)	43/147 (29 %)	61/178 (34 %)
# litters affected/evaluated (%)	55 – 96 % (74.8 %)	15/22 (68 %)	14/20 (70 %)	16/21 (76 %)	22/25 (88 %)
Rudimentary cervical rib with no cartilage					
# foetuses affected/evaluated (%)	0 – 6.6 % (2.0 %)	1/158 (0.6 %)	2/151 (1.3 %)	2/147 (1.4 %)	9/178 (5.1 %)
# litters affected/evaluated (%)	0 – 29 % (10.8 %)	1/22 (4.5 %)	2/20 (10 %)	2/21 (9.5 %)	8/25* (32 %)

Wilcoxon test (one-sided; for foetus/litter incidence); Fisher's exact test (one-sided; for litter incidence): *p<0.05, **p<0.01

[&]study mean not given in the report but calculated by DS

An increase in total incidence of soft tissue variations might be suspected in all treated groups but all group incidences were well within the historical control range and not far from the mean foetal and litter incidences (Table 58). In contrast, an outstandingly low incidence of such anomalies was noted in the control group. In addition, at least the litter incidence did not exhibit a clear dose response. The same considerations apply for the specific findings of dilated ureter or dilated renal pelvis.

Table 65: Developmental toxicity study in the rat (TOX2000-730: 1999): Summary of soft tissue variations against the background of historical control data provided by ASB2019-10765: 2019

Findings	Historical control range (min-max, study mean in brackets[&])	Dose level (mg/kg bw/day)			
		0	10	25	50
Total soft tissue variations					
# foetuses affected/evaluated (%)	6.7 – 33 % (15.1 %)	8/148 (5.4 %)	18/140 (13 %)	21/136 (15 %)	31/165 (19 %)
# litters affected/evaluated (%)	30 – 80 % (55.6 %)	6/22 (27 %)	12/20* (60 %)	9/21 (43 %)	15/24*[$] (63 %)
Dilated renal pelvis					
# foetuses affected/evaluated (%)	6.7 – 33 % (16.1 %)	8/148 (5.4 %)	16/140 (11 %)	20/136 (15 %)	31/165 (19 %)
# litters affected/evaluated (%)	30 – 80 % (58.1 %)	6/22 (27 %)	10/20 (50 %)	9/21 (43 %)	15/24* (63 %)
Dilated ureter					
# foetuses affected/evaluated (%)	0.5 – 6.7 % (2.1 %)	0/148 (0 %)	4/140 (2.9 %)	1/136 (0.7 %)	5/165 (3.0 %)
# litters affected/evaluated (%)	3.2 – 28 % (12 %)	0/22 (0 %)	4/20* (20 %)	1/21 (4.8 %)	3/24 (13 %)

Wilcoxon test (one-sided; for foetus/litter incidence); Fisher's exact test (one-sided; for litter incidence): *p<0.05, **p<0.01

[$] In one litter, the only fetus had a malformation (macroglossia). Therefore, this litter was excluded from evaluation of variations.

[&]study mean not given in the report but calculated by DS

On the other hand, the developmental effects observed in the prenatal developmental toxicity study in rabbits (TOX2000-731: 1999) is considered as severe such that classification for developmental toxicity needs to be considered. In this study, pronounced maternal toxicity in terms of a severely depressed food intake at the beginning of treatment and initial body weight losses (see Table 60) was observed at all doses (i.e. 5, 10 and 20 mg/kg bw/day), and the maternal NOAEL was set at < 5 mg/kg bw/day. A subsequent maternal toxicity study (TOX2001-471: 2001) was conducted in pregnant rabbits using lower doses (1, 3 and 5 mg/kg bw/day), and this study identified a maternal NOAEL of 3 mg/kg bw/day (see Table 54g). It must be emphasised, however, that the range of foetal parameters examined in this study was very limited.

Developmental toxicity to rabbit foetuses was manifested at maternally toxic dose levels. There was a strong increase in post-implantation loss and a related decrease in the mean number of viable foetuses per litter at the top dose level of 20 mg/kg bw/day. At the mid-dose of 10 mg/kg bw/day, post-implantation loss was less pronounced and statistical significance was lacking. However, taking into consideration the dose-dependent increase in post-implantation loss as well as the occurrence of complete litter loss due to resorption in three high and two mid-dose females that is above the range of the historical control data (see tables below), the

adverse developmental effects observed also at 10 mg/kg bw/day appears likely to be treatment-related.

Table 66: Developmental toxicity study in the rabbit (TOX2000-731: 1999): Reproductive findings

Parameter	Dose level (mg/kg bw/day)			
	0	**5**	**10**	**20**
Number of pregnant females at terminal sacrifice	24	24	22	25
Implantation sites (mean per litter)	7.4	6.6	6.9	7.0
Postimplantation loss	6.2 %	10.2%	17.8%	38.6%**
Total resorptions (mean per litter)	0.5	0.6	1.3	2.7**
Early resorptions (mean per litter)	0.4	0.5	1.2	2.6**
Does with viable foetuses / total number of live foetuses	24 / 166	24 / 145	20 / 123	22 / 107
Resorption of the total litter, number of does affected	-	-	2	3
Live foetuses (mean per litter)	6.9	6.0	6.2	4.9**

Dunnett's test *p<0.05, **p<0.01

Table 67: Historical control data (Feb. 1993 – Nov. 2003; n = 23) for the same rabbit strain from studies in the same laboratory (ASB2019-10765: 2019)

	Minimum	**Maximum**	**Mean**
Postimplantation loss (%)	4.6	20.1	10.7
Early resorptions (mean per doe)	0.2	0.9	0.4
Resorptions of the total litter, number of does affected	0	1	ca. 0.2 (occurring in 5 out of 23 studies)
Live foetuses (mean per dam)	5.7	7.8	6.6

The incidence of external or soft tissue malformations or variations of any type was very low and was not affected by treatment. The only remarkable visceral finding was a septal defect in the heart of three foetuses from three litters at the mid-dose level. There was zero incidence of this malformation both in the control and high dose groups and just a single case at the low dose level. One plausible explanation for the lack of this visceral finding in the high dose group might be that the effect was masked by the higher embryotoxicity at this dose.

There was an apparent increase in skeletal malformations at the high dose level (see table below). The absolute number of affected foetuses was increased by 50% over the control incidence. This increment resulted in a higher mean percentage of affected foetuses per litter that was clearly outside of the historical control range. A very slight exceedance of the upper edge of the historical control data was also noted for the mid-dose group. Although statistical significance was not observed (using Wilcoxon test) between the control and any of the dose groups, analysis using the Cochrane Armitage Trend Test revealed a significant positive trend.

The observed skeletal malformations comprise mainly two findings: absent and misshapen lumbar vertebrae. The increase in these two malformations was confined to the top dose level and the incidence of absent lumbar vertebrae was outside the historical control range.

Table 68: Developmental toxicity study in the rabbit (TOX2000-731: 1999): Skeletal malformations in foetuses including historical control data from 7 studies performed 1995 and 1997 in the same laboratory and the same strain

Malformations	Historical control range, study mean in brackets	Dose level (mg/kg bw/day)			
		0	5	10	20
Total foetal skeletal malformations					
# foetuses affected/evaluated (%)	0 – 3.5 % (1.9 %)	6/166 (3.6 %)	4/145 (2.8 %)	5/123 (4.1 %)	9/107 (8.4 %)
# litters affected/evaluated (%)	0 – 17.6 % (9.3 %)	6/24 (25 %)	4/24 (17 %)	4/20 (20 %)	7/22 (32 %)
Absent lumbar vertebrae					
# foetuses affected/evaluated (%)	0 – 0.9 % (0.3 %)	1/166 (0.6 %)	1/145 (0.7 %)	1/123 (0.8 %)	4/107* (3.7 %)
# litters affected/evaluated (%)	0 - 5.9 % (1.7 %)	1/24 (4.2 %)	1/24 (4.2 %)	1/20 (5 %)	4/22* (18.2 %)
Misshapen lumbar vertebrae					
# foetuses affected/evaluated (%)	0 – 2.2 % (0.4 %)	1/166 (0.6 %)	1/145 (0.7 %)	0/123 (0 %)	2/107 (1.9 %)
# litters affected/evaluated (%)	0 – 7.1 % (1.7 %)	1/24 (4.2 %)	1/24 (4.2 %)	0/20 (0 %)	2/22 (9.1 %)

* significant positive trend according to Cochrane Armitage Trend Test $p<0.05$

10.8.6 Comparison with the CLP criteria

Please refer to Section 10.10.9 for the comparison.

10.8.7 Adverse effects on or via lactation

Table 69: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Two-generation reproduction toxicity study; OECD 416 Wistar rats; M/F 25/sex/group GLP-compliant Deviation: From day 14 after parturition onwards, food consumption was not determined since the pups themselves began to consume considerable amounts of solid food. This deviation from guideline recommendations did not alter the validity of the results obtained.	Pyraclostrobin (purity 98.7%) 0, 25, 75, 300 ppm Daily test substance intake 0, 2.7, 8.2, 32.6 mg/kg bw/day (calculated for the whole study period with all phases, for all groups and both sexes) Dietary exposure F_0: Treatment started 74 days before mating and lasted until after weaning of F_1 pups.	No indication of effect on or via lactation (e.g. no difference in lactation index in F_0 or F_1 generation)	TOX2000-729: 1999

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	F_1: Exposed continuously from their growth into adulthood until or up to 16 hours before sacrifice (i.e. after weaning of F_2 pups).		

Table 70: Summary table of human data on effects on or via lactation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
N/A				

Table 71: 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
N/A				

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

The two-generation reproduction study in rats performed in accordance with OECD Guideline 416 (TOX2000-729: 1999) showed no indication of effects on or via lactation.

10.8.9 Comparison with the CLP criteria

The following criteria for classification for reproductive toxicity are given in the CLP Regulation:

Table 72: Comparison with the CLP criteria for reproductive toxicity

CLP criteria (Proposed classification/rationale in bold)
Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on development in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development and - the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

There are no appropriate epidemiological studies available on developmental effects in humans. Hence, classification for Category 1A according to the CLP Regulation is not possible. Furthermore, the two-generation reproduction toxicity study in rats does not warrant any classification and labelling for reproductive effects or lactation. Likewise, the very few findings in rat foetuses, in the presence of overt maternal toxicity in the developmental study, do not support a need for classification and labelling.

However, the higher early resorption rate (resulting in complete embryolethality), post-implantation losses and the increase in skeletal malformations in rabbit foetuses should be considered with regard to classification for developmental toxicity. In rabbits, there was clear evidence of an adverse effect on development as proven by alterations of gestational parameters (post-implantation losses/early resorptions) and an increase in skeletal malformations (TOX2000-731: 1999). At the mid-dose level, higher incidences of septum defects were reported. The increase in resorptions, i.e., intrauterine death of the developing organism, is a major manifestation of developmental toxicity and would be most likely already sufficient for classification. The malformations, i.e., structural abnormalities, provide further support that there is a need for classification and labelling even though the observed incidences did not gain statistical significance by group-wise comparison but only a positive trend was observed.

While maternal toxicity was observed in treated does, it is considered not of sufficient severity to induce the observed foetal findings by unspecific mechanism. Additionally, it was not demonstrated that the type and extent of maternal findings (especially alterations of body weight and feed intake) usually induce the observed foetal findings in the used strain of Himalayan rabbits in the performing laboratory.

In principle, Category 1B might be considered, but the developmental effects have been observed only in the presence of maternal toxicity. In the ECHA's "Guidance on the Application of CLP Criteria" (Version 5.0 – July 2017), the following is stated: "*Classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally-mediated mechanism has been demonstrated.*" The latter is not the case for pyraclostrobin. However, as it is further mentioned in the guidance that "*In such a case, classification in Category 2 may be considered more appropriate than in Category 1.*" It seems that this approach would be most appropriate for pyraclostrobin taking into account that effects warranted for classification were seen only in rabbits but not in rats and skeletal malformations, even in rabbits, were few.

10.8.10 Conclusion on classification and labelling for reproductive toxicity

Considering the severity of the observed developmental effects, Category 2 for developmental toxicity (H361d, "Suspected of damaging the unborn child") is proposed.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The following studies were presented in the CLH report for the evaluation of reproductive toxicity of pyraclostrobin

- A two-generation reproduction toxicity study in rats
- A one-generation reproduction toxicity study in rats (dose-range finding study for the above)
- A prenatal developmental toxicity (PNDT) study in rats
- A PNDT study in rabbits
- A maternal toxicity study in rabbits (i.e., a subsequent PNDT study in rabbits but with a focus on investigating maternal toxicity; foetuses were only removed, counted and weighed. There was no evidence of gross malformations).
- The chronic toxicity study in Wistar rats (histopathological effects observed in testes were reported but these were regarded as not treatment-related and secondary to the atrophy and/or the spontaneous Leydig cell tumours common in this strain).

Adverse effects on sexual function and fertility

Based on no relevant adverse effects having been observed in the two-generation reproduction toxicity study (or the dose-range finding one-generation reproduction toxicity study), the DS proposed no classification for adverse effects on sexual function and fertility, and for adverse effects on or via lactation.

Adverse effects on development

The DS proposed Repr. 2; H361d for adverse effects on development observed in the PNDT study in rabbits. Increased post-implantation losses (total and early resorptions) and increased skeletal malformations were observed in the high dose group (20 mg/kg bw/d). While severe maternal toxicity was observed in the treated does, the DS did not consider it to be of sufficient severity to induce foetal findings by a secondary non-specific mechanism.

Comments received during consultation

Three MSCAs and a Company/manufacturer commented. All 3 MSCAs supported the DS proposal but recommended to assess if there was a correlation between the severity of maternal toxicity in individual does and the developmental effects. The DS in response provided information on some of the individual does and concluded that there was no correlation (see information further below in the Assessment section).

The Company/manufacturer commented that should RAC consider classification necessary, then Category 2 is most appropriate (i.e., they supported DS proposal). It provided further input on the developmental findings including additional historical control data and the

critical comments concern the severity of maternal toxicity and on the incidence of skeletal malformations (see information further below in the Assessment section).

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

In a two-generation reproduction toxicity study (OECD TG 416, GLP-compliant and no significant deviations affecting the validity of the study[1]), pyraclostrobin (purity: 98.7%) was administered via the diet to Wistar rats (25/sex/group) at 25, 75 and 300 ppm (corresponding to 2.7, 8.2 and 32.6 mg/kg bw/d) (TOX2000-729: 1999).

Parental toxicity was manifested as reduced food consumption (by around 5%) in high dose males and females, slightly decreased body weight/body weight gain in high dose males and females (mostly ≤ 5% and not statistically significant); relative kidney weight in F0 and F1 males statistically significantly increased by 5.6 or 7.0%, respectively. Given the mild parental toxicity, RAC notes that the maximum tolerated dose was not reached in this study.

No treatment-related adverse effects on sexual function and fertility were reported in this study.

As a dose-range finder for the above, a one-generation reproduction toxicity study was performed with pyraclostrobin (purity: 97.09%) that was similar to OECD TG 415 and was GLP-compliant but had significant deviations regarding the number of animals (10/sex/group instead of 20/sex/group) and exposure duration (F0 generation treated for 45 days premating instead of 70 days) (ASB2017-5538: 2002). The dose levels were 200, 400 and 600 ppm, corresponding to:

Males: 20.5, 39.9, 59.1 mg/kg bw/d

Females

- premating: 21.3, 42.5, 60.4 mg/kg bw/d
- gestation: 18.3, 35.0, 53.2 mg/kg bw/d
- lactation (post-natal day (PND) 1-14): 29.0, 51.9, 80.2 mg/kg bw/d

Parental toxicity was manifested as:

- statistically significant and dose-related reductions in food consumption (up to 10% in males and 15% in females at some stages of the study) and statistically significant decreases in body weight/bw gain (up to 10 – 20% in males and 6 – 18% in females) at the mid- and high doses;
- anaemia in mid- and high dose males and females (<5% decrease in haemoglobin);

[1] From day 14 after parturition onwards, food consumption was not determined since the pups themselves began to consume considerable amounts of solid food.

- organ weight changes in parental males: statistically significant decreases of absolute liver (up to 15% reduction) and kidney (by ca 13%) weights as well as the statistically significant increases of relative testes (by up to 21%) and epididymis weights (+22 %) in mid- and high dose groups which were not accompanied by respective changes in relative (liver & kidney) or absolute (testes & epididymis) weights or by any gross lesions in these organs. The only gross pathology finding was a thickening of the wall of the duodenum in all high dose males.

No treatment-related adverse effects that are relevant for classification for sexual function and fertility were reported in this study.

Since no relevant adverse effects were observed in either one- or two-generation studies, RAC agrees with the DS and concludes that **pyraclostrobin warrants no classification for adverse effects on sexual function or fertility.**

Adverse effects on development

In the two-generation study, the pup body weight was statistically significantly reduced at the high dose (on PND 21: - 10% in F1 males and -12% in F1 females; -15% in F2 males and -13% in F2 females). RAC considers that the pup body weight decreases on PND 21 may partly be due to pups starting to consume the diet from PND 14. However, decreased pup body weight was also observed in F2 animals (but not F1) on PND 7 by ca. 10%.

In F1 animals vaginal opening was delayed by 1.6 days at the high dose which was due to lower body weight gain. "*Both, control and high-dose female F1 animals, had the same body weight (103.0 g ± 10.9 and 103.0 g ± 9.3, respectively) at vaginal opening, however, the high dose rats achieved this weight 1.6 days later due to the lower body weight gain*" (from the attachment submitted with the comment by the company).

Table*: Pup developmental findings in the two-generation study (TOX2000-729: 1999) (Table 58 in the CLH report)*

Parameter	Dose level (ppm)			
	0	**25**	**75**	**300**
F1 - Body weight, day 21, M / F (g)	52.8 / 51.4	53.7 / 51.3	52.8 / 50.3	**47.4**/ 45.2****
F1 - Vaginal opening (days)	31.7	32.1	32.4	**33.3****
F2 - Body weight, day 7, M / F (g)	15.2 / 14.5	14.9 / 14.6	14.7 / 14.2	**13.5**/ 13.1****
F2 - Body weight, day 21, M / F(g)	52.0 / 49.8	52.6 / 50.2	51.4 / 48.9	**45.0**/ 43.5****

Dunnett's test **p< 0.01

In the one-generation study, there was a dose-related statistically significant decrease in pup body weight gain over PND 4 to 21 in all treated groups (up to ca. -40% at the high dose). Although not specifically reported, the pups may have started consuming the diet from PND 14 also in this study and the decrease in body weight on PND 21 may partly be due to this. Nevertheless, pup body weight showed a dose-dependent decrease of up to -33% at the high dose already on PND 14. There was no statistically significant change in body weight gain of the parental females during lactation. Therefore, RAC considers that the decrease in pup body weight (up to -33% on PND 14) supports classification.

***Table**: Pup body weight changes in the one-generation study (ASB2017-5538: 2002) (adapted from Table B.6.6-2 in RAR Vol. 3CA - B.6)*

Dose [ppm]		**0**	**200**	**400**	**600**
Male pup weight	[g]				
- day 1	[g]	6.6	6.5	6.6	6.1
- day 4 - pre cull	[g]	9.6	9.1	9.2	**8.0****
- day 4 - post cull	[g]	9.6	9.1	9.1	**8.2***
- day 7	[g]	15.3	13.9	13.5	**11.6****
- day 14	[g]	30.3	**25.8****	**25.1****	**20.3****
- day 21	[g]	50.2	**41.9****	**39.7****	**31.4****
Male body weight gain					
- day 4 to 21	[g]	40.6	**32.8****	**30.5****	**23.4****
[%]			**-9.2**	**-14.9**	**-42.4**
Female pup weight	[g]				
- day 1	[g]	6.4	6.3	6.3	**5.8***
- day 4 - pre cull	[g]	9.4	9.0	9.1	**7.8***
- day 4 - post cull	[g]	9.4	9.1	9.1	**7.7***
- day 7	[g]	14.7	14.0	13.3	**11.3****
- day 14	[g]	29.8	26.4	**24.6****	**20.1****
- day 21	[g]	47.7	**42.0***	**38.9****	**31.1****
Female body weight gain					
- day 4 to 21	[g]	38.4	**33.0***	**29.8****	**23.3******
[%]			**-14.1**	**-22.4**	**-39.3**
Statistics: *: p≤0.05, **: p≤0.01					

In a prenatal developmental toxicity study in Wistar rats (OECD TG 414, GLP-compliant and no deviations), pyraclostrobin (purity: 98.9%) was administered via gavage to 25 dams/group during gestation day (GD) 6 – 19 at 10, 25 and 50 mg/kg bw/d (TOX2000-730: 1999).

Maternal toxicity was manifested as statistically significantly reduced food consumption (mid dose: -14%; high dose: -27%) on the first days of treatment (GD 6 – 8) resulting in a statistically significantly lower corrected body weight gain in both groups (-22% and -45%, respectively) (see Table 59 in the CLH report).

No treatment related malformations were observed. At the high dose, increases in the incidences of skeletal variations (rudimentary cervical rib with no cartilage; no dose-

response relationship, slightly above the maximum value of the historical control[2] range) and soft tissue variations (dilated renal pelvis; no dose-response relationship, within the range of historical controls) were observed (see Table 64 in the CLH report). RAC considers that the variations observed in this study do not merit classification.

In a prenatal developmental toxicity study in Himalayan rabbits (OECD TG 414, GLP-compliant and no deviations), pyraclostrobin (purity: 98.9%) was administered via gavage to 25 does/group during GD 7 – 28 at 5, 10 and 20 mg/kg bw/d (TOX2000-731: 1999).

Maternal toxicity was severe and manifested as statistically significantly lower food intake (by 64, 79, and 89% at the low-, mid-, and high dose) on the first two days of dosing, resulting in severe initial body weight losses in all dose groups.

Similar findings were observed in the follow-up maternal toxicity study in Himalayan rabbits (TOX2001-471: 2001). Statistically significant decreases in initial food consumption on the first two days of dosing were noted at the mid dose (3 mg/kg bw/da; -26%) and at the high dose (5 mg/kg bw/d; -41%) (see Table 61 in the CLH report).

***Table**: Maternal findings in the prenatal developmental toxicity study in rabbits (TOX2000-731: 1999) (Table 60 in the CLH report)*

Parameter	Dose level (mg/kg bw/day)			
	0	**5**	**10**	**20**
Food consumption; day 7-8 (g/animal/day)	98.1	**35.7****	**20.4****	**10.5****
Body weight change; day 7-9 (g)	-3.8	**-43.8****	**-85.5****	**-146.3****
Body weight change; days 7-28 (g)	191.5	131.7	**116.0***	**44.1****
Body weight; day 29 (g)	2961	2807	2851	**2748****
Corrected body weight gain over treatment period (g)	-135.7	-142.4	-132.9	-146.8
Gravid uterus (g)	352.6	302.6	**271.2***	**209.6****

Dunnett's test *p<0.05, **p<0.01

Developmental toxicity was manifested as:

- increased post-implantation losses (total and early resorptions) at the mid dose (17.8% vs 6.2% in controls; not statistically significant, below the maximum value of historical control[3] range) and at the high dose (38.6% vs 6.2% in controls; statistically significant, outside historical control range); 2 does in the mid dose and 3 does in the high dose had resorption of the total litter (vs. none in controls; outside the historical control range); live foetuses were statistically significantly reduced at the high dose (4.9 vs 6.9 in controls).
- at the high dose, an increase in total frequency of skeletal malformations (32% vs 25% in controls; not statistically significant but outside historical control[4] range (0 – 17.6%) (NB: also the controls were outside the HCD range)). The skeletal

[2] Twenty-nine PNDT studies in Wistar rats from the same laboratory and animal supplier performed between 1993 and 1999.

[3] Twenty-three PNDT studies in Himalayan rabbits from the same laboratory performed between 1993 and 2003.

[4] Seven PNDT studies in Himalayan rabbits from the same laboratory performed between 1995 and 1997.

malformations of concern are in particular absent lumbar vertebrae (18.2% vs 4.2% in controls; statistically significant in the Cochrane Armitage Trend Test; outside the historical control range (0 - 5.9 %)).

***Table**: Developmental findings in the prenatal developmental toxicity study in rabbits (TOX2000-731: 1999) (adapted from Tables 66 and 67 in the CLH report)*

Parameter	**Historical control data,** where available **Mean (min – max)**	**Dose level (mg/kg bw/day)**			
		0	**5**	**10**	**20**
Number of pregnant females at terminal sacrifice		24	24	22	25
Implantation sites (mean per litter)		7.4	6.6	6.9	7.0
Post-implantation loss	10.7% (4.6 – 20.1%)	6.2%	10.2%	**17.8%**	**38.6%****
Total resorptions (mean per litter)		0.5	0.6	**1.3**	**2.7****
Early resorptions (mean per litter)	0.4 (0.2 – 0.9)	0.4	0.5	**1.2**	**2.6****
Does with viable foetuses / total number of live foetuses		24 / 166	24 / 145	20 / 123	22 / 107
Resorption of the total litter, number of does affected	0.2# (0 – 1)	-	-	**2**	**3**
Live foetuses (mean per litter)		6.9	6.0	6.2	**4.9****

Dunnett's test *p<0.05, **p<0.01
Occurring in 5 out of 23 studies

***Table**: Skeletal malformations in the prenatal developmental toxicity study in rabbits (TOX2000-731: 1999) (adapted from Table 68 in the CLH report)*

Malformations	**Historical control range**, study mean in brackets	**Dose level (mg/kg bw/day)**			
		0	**5**	**10**	**20**
Total foetal skeletal malformations					
# litters affected/evaluated (%)	0 – 17.6 % (9.3 %)	6/24 (25 %)	4/24 (17 %)	4/20 (20 %)	7/22 (32 %)
Absent lumbar vertebrae					
# litters affected/evaluated (%)	0 - 5.9 % (1.7 %)	1/24 (4.2 %)	1/24 (4.2 %)	1/20 (5 %)	**4/22* (18.2 %)**
Misshapen lumbar vertebrae					
# litters affected/evaluated (%)	0 – 7.1 % (1.7 %)	1/24 (4.2 %)	1/24 (4.2 %)	0/20 (0 %)	2/22 (9.1 %)

*** statistically significant positive trend according to Cochrane Armitage Trend Test p<0.05**

Maternal toxicity vs developmental toxicity

The DS expressed their view on maternal toxicity and post-implantation loss in their response to comment No. 12, as follows (excerpt from the RCOM):

> *"Significant reduction in food consumption and concomitant body weight loss observed early in treatment [i.e., gestation day (GD) 7-9] could have contributed to the severe reproductive outcomes such as increased early resorption and post-implantation loss. However, this might not be the only cause for the observed reproductive outcomes as the data of the individual does showed no correlation between reduced food consumption/body weight gain and the severity of reproductive outcomes.*
>
> *In particular, while all the does at the high dose group (20 mg/kg bw/d) consumed significantly less feed than the control group and exhibited marked body weight loss between GD 7-9, not all of the does experienced significant post-implantation loss. For example, doe nos. 75, 76, 78, 86, and 97 with minimal food consumption (≤ 13.5 g/animal/d between GD 7-8) and dramatic body weight loss (up to -184 g between GD 7-9) had minimal to no post-implantation loss (up to ≤ 12.5%). A similar observation was made also in the mid-dose group (10 mg/kg bw/d). To further demonstrate the lack of correlation, doe no. 73 at the mid-dose group with 100% post-implantation loss exhibited only a slight body weight loss (-55 g) between GD 7-9.*
>
> *This suggests that maternal toxicity indicated by reduced food consumption and body weight gain might not be the only factor contributing to the post-implantation loss in rabbits. It is our opinion that the degree of maternal toxicity observed is considered not severe enough to trigger such adverse reproductive outcomes like resorption of the total litter."*

The DS expressed their view on maternal toxicity and skeletal malformations in their response to comment No. 12, as follows (excerpt from the RCOM):

> *"Regarding the degree of maternal toxicity observed in the does with lumbar vertebrae malformations found in their litters, there is no clear relationship between maternal toxicity and lumbar vertebrae malformations.*
>
> *Five does (no. 76, 87, 90, 92 and 93) had at least one foetus with lumbar vertebrae malformations (misshapen or absent). These does did not exhibit any clinical signs of toxicity during gestation and nothing abnormal was detected during necropsy. Three of them (76, 90 and 92) had no post-implantation loss, whereas does 87 and 93 had a post-implantation loss of 25 % and 16.7 %, respectively.*
>
> *By study termination, does nos. 76 and 90 had more than 10 % decrease in terminal body weight compared to the average terminal body weight of the control group, whereas does nos. 87 and 93 had a slight decrease (5-7 %) in body weight. Doe 92 had a terminal body weight higher than the average terminal body weight of the control.*
>
> *Altogether, the lumbar vertebrae malformation findings do not appear to be directly correlated with maternal toxicity."*

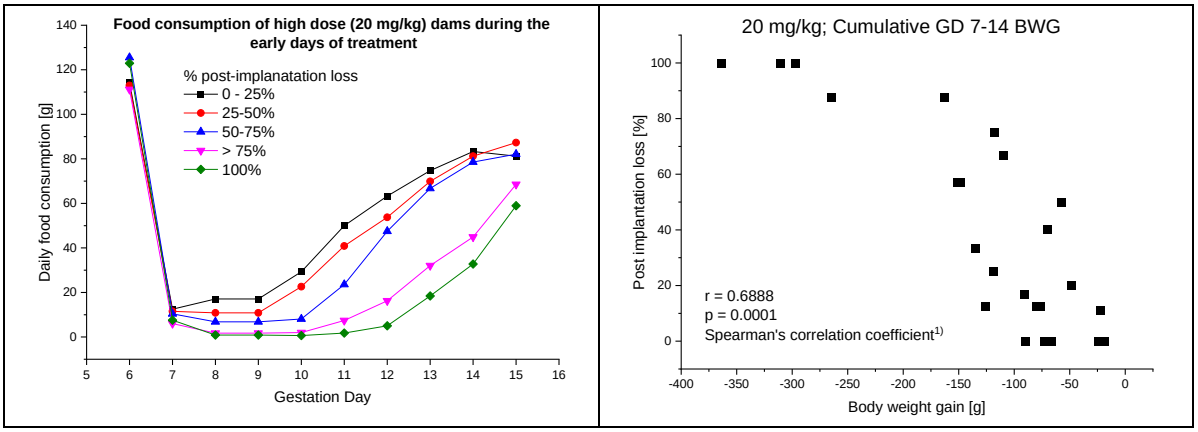
Studies on impact of feed restriction on developmental toxicity are available in New Zealand

White rabbits, for e.g., Cappon *et al*., 2005, and Matsuoka *et al*., 2006 (referred to in the attachment submitted with the comment by the company).

In Cappon *et al*. (2005), feed restriction to 15 g/d during GD 7 – 19 led to abortions in 6 of 15 does (vs. none in controls) but no malformations were observed.

In Matsuoka *et al*. (2006), feed restriction to 20 g/d during GD 6 – 18 led to no abortions in the 8 does in the group but there was an increase in post-implantation loss (14.3% vs. 3.1% in controls) and increase in number of does with resorptions (75% vs 12.5% in controls).

In the absence of feed restriction data on Himalayan rabbits, RAC has considered the data on New Zealand White rabbits. The above two studies indicate that severe reduction in food consumption (20 g/d) including the initial days of gestation may lead to abortions or post-implantation loss in rabbits. In the current PNDT study with pyraclostrobin in Himalayan rabbits, there was severe reduction in food consumption in the mid dose (20.4 g/d) and the high dose (10.5 g/d). At high dose, the five does with the most severe body weight gain loss (>150 g) in the group during GD 7 - 14 had the highest post-implantation loss (>80%). See the right panel of the figure below. Therefore, there is a correlation between the maternal toxicity and the post-implantation loss in these. However, similar to that indicated by the DS in the RCOM (quoted above), there was no correlation in the does with body weight gain loss <150 g. Thus, RAC agrees with the DS that the maternal toxicity might not be the only factor contributing to the post-implantation loss in the rabbit PNDT study with pyraclostrobin, and the increased post-implantation loss supports classification.



1) Spearman C. (1904). "The proof and measurement of association between two things". American Journal of Psychology. 15 (1): 72–101

Figure: Correlation of the extent and duration of food consumption respectively body weight loss during GD 7 to 14 and post-implantation loss in pregnant rabbits treated with 20 mg/kg of pyraclostrobin (copy of figure 4 in the attachment submitted with the comment by the company)

In line with the Cappon *et al*. (2005) conclusion that severe feed restriction did not cause malformations in rabbits, the DS in the RCOM (quoted above) presented that there is no correlation between maternal toxicity (reduced feed consumption and body weight (gain) loss) and malformations in the rabbit PNDT study with pyraclostrobin. The increased incidence of skeletal malformations (absent lumbar vertebrae) at the high dose in this study did not gain statistical significance by group wise comparison but a statistically

significant positive trend (Cochrane Armitage Trend Test $p<0.05$) was observed. Therefore, RAC considers that the increased incidence of absent lumbar vertebrae supports classification.

Overall, RAC considers that the following adverse effects provide "some evidence" for developmental toxicity of pyraclostrobin:

- decrease in pup body weight in the one-generation study (up to -33% on PND 14) and the two-generation study (ca. -10% on PND 7 in F2 animals),
- increased post-implantation loss (not completely attributed to maternal toxicity) in the rabbit PNDT study; and
- increased incidence of skeletal malformations (absent lumbar vertebrae) gaining significance by trend test in the rabbit PNDT study.

Therefore, RAC agrees with the DS and concludes that **pyraclostrobin warrants classification as Repr. 2; H361d**.

Adverse effects on or via lactation

Classification for effects on or via lactation can be assigned based on:

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

There is no data on pyraclostrobin fulfilling criteria 'a' or 'c' above. With regard to criterion 'b', adverse effect in the offspring (decreased body weight) during the lactation period was observed in the one-generation study. However, there is no clear evidence of whether this effect is due to pre-natal exposure or due to transfer in the milk or an adverse effect on the quality of the milk.

Therefore, RAC agrees with the DS and concludes that **pyraclostrobin warrants no classification for adverse effects on or via lactation.**

10.9 Specific target organ toxicity-single exposure

Table 73: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Acute oral toxicity OECD 401 Rat (Wistar), M/F, 5/sex/dose GLP-compliant No deviations	Pyraclostrobin (purity: 98.5 %) 2000 and 5000 mg/kg bw Single dose Observed up to 15-d post-dosing	No macroscopic findings at necropsy at the end of the observation period.	TOX2000-709: 1998
Acute dermal toxicity OECD 402 Rat (Wistar), M/F, 5/sex/dose GLP-compliant No deviations	Pyraclostrobin (purity 98.2 %) 2000 mg/kg bw Single exposure for 24 h, Semi-occlusive application 14-d post-application observation period	No pathological findings were detected in the animals at necropsy.	TOX2000-710: 1998
Acute inhalation toxicity OECD 403 Rat (Wistar), M/F, 5/sex/dose GLP-compliant No significant deviations that would affect the study validity.	0, 0.31, 1.07, 5.3 mg/L air (vehicle: acetone) 4 h, nose-only 14-d post-exposure observation period	Necropsy of the mid-concentration (1.07 mg/L) animals showed agonal congestive hyperaemia. No further macroscopic pathological findings were noted in animals exposed to the low concentration examined at the end of the study or in the high concentration-exposed animals that died during the study.	TOX2000-711: 1997
Acute inhalation toxicity OECD 403 Rat (Wistar), M/F, 5/sex/dose GLP-compliant No deviations	0, 0.52, 0.65, 0.85 mg/L air (vehicle: acetone) 4 h, nose-only 14-d post-exposure observation period	Gross necropsy of the animals that died during the study revealed mainly red discolorations of the lungs. In most of the deceased cases, all lung lobes were affected. One mid-concentration male also displayed lung oedema in all lobes.	ASB2008-5020: 2002

Table 74: Summary table of human data on STOT SE

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
Clinical incident log at occupational site	Pyraclostrobin (presumed; purity unknown)	The affected people became exposed to pyraclostrobin-containing products in the course of production, transportation, formulation and packaging but mostly in combination with other active ingredients and/or products. It is not clear whether pyraclostrobin was the cause for these effects and whether exposure was according to good agricultural practice.	Some cases of slight irritation of the eyes, skin and mouth and/or intoxication (indisposition, headache, ague, fatigue, aching muscles, vomiting, drowsiness, dizziness, adynamic feet, breathing difficulties) have been registered in the internal clinical incident log.	Self-reported information from the applicant
Poisoning incident report	Pyraclostrobin (purity unknown)	Five independent poisoning events of pyraclostrobin reported with a total of 33 persons affected. All events occurred in the US state of Iowa.	All affected cases were categorised as being of low to moderate severity. The patients complained about irritation and pain of the upper respiratory tract as well as nausea, headache, eye pain, weakness, dizziness, and chest pain.	ASB2015-8357: Gergely et al., 2007

Table 75: Summary table of other studies relevant for STOT SE

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
N/A				

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

From the acute toxicity animal studies (see Sections 10.1-10.3), organ findings were mostly restricted to the lungs after acute inhalation toxicity, but they were associated with mortality. Most of the surviving animals did not exhibit any specific target organ toxicity. Therefore, the observed findings from these animal studies were not considered suitable for STOT SE classification.

On the other hand, there are several reports of human poisoning cases of pyraclostrobin as described below that were considered relevant for STOT SE classification.

Some cases of slight irritation of the eyes, skin and mouth and/or intoxication (indisposition, headache, ague, fatigue, aching muscles, vomiting, drowsiness, dizziness, adynamic feet, breathing difficulties) have been registered in the internal clinical incident log from the applicant. The affected people became exposed to pyraclostrobin-containing products in the course of production, transportation, formulation and packaging but mostly in combination with other active ingredients and/or products. However, it remains unclear whether pyraclostrobin was the cause for these effects and whether exposure was according to good agricultural practice. No details of these case reports have been made available for further evaluation.

A publication by Gergely et al. (ASB2015-8357: Gergely et al., 2007) described five independent events of pyraclostrobin poisoning, which all occurred in the US state of Iowa, with a total of 33 persons affected. All cases were categorised as being of low to moderate severity. The patients complained irritation and pain of the

upper respiratory tract as well as nausea, headache, eye pain, weakness, dizziness, and chest pain.

In the first event, 27 migrant workers (20 men and 7 women) of Hispanic ethnic background were accidentally exposed when working in the crops to off-target drift of pyraclostrobin after spraying an adjacent field from a crop-duster plane. Some workers reported feeling wet droplets on their skin and seeing mist coming from the aircraft. Although all of them received on-site skin decontamination by a hazardous materials team before being transported to an emergency department for observation, 26 of them complained of upper respiratory tract pain or irritation. Another common symptom was chest pain (20 patients). Three patients had nausea, and one patient each had pruritis, skin redness, eye pain, weakness, headache, or exhibited dizziness.

There were three other events of exposure to pyraclostrobin due to off-target drift from nearby aerial applications. In total, five people were affected. They were exposed when riding a motorcycle near a field that was just under treatment or by spray drifting to their home yard. Symptoms reported were headache, eye pain partially associated with conjunctivitis and dizziness.

The fifth reported event was also related to aerial spraying, but the affected case was rather the crop-duster pilot, who was dermally exposed to the liquid fungicide when his plane crashed during take-off and the fungicide spilled. The pilot exhibited chemical burns but reported no respiratory symptoms. The case was considered to be of moderate severity, and the man had to stay in hospital for two days.

10.9.2 Comparison with the CLP criteria

Table 76: Comparision with the CLP criteria for STOT SE

Toxicological result (pyraclostrobin)	CLP criteria
No animal data indicating non-lethal STOT effects after acute exposure Human data from poisoning incidences of pyraclostrobin indicated symptoms of irritation and pain of the upper respiratory tract as well as nausea, headache, eye pain, weakness, dizziness, and chest pain.	STOT SE 1: Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure Substances are classified in Category 1 for specific target organ toxicity (single exposure) on the basis of: a. reliable and good quality evidence from human cases or epidemiological studies; or b. observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations. STOT SE 2: Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following single exposure Substances are classified in Category 2 for specific target organ toxicity (single exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. In exceptional cases, human evidence can also be used to place a substance in Category 2. STOT SE 3: Transient target organ effects – This category only includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2 indicated above. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function.

10.9.3 Conclusion on classification and labelling for STOT SE

STOT SE 3, "May cause respiratory irritation", H335.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

No significant target organ findings were reported in the acute toxicity studies with pyraclostrobin except in the lungs in the acute inhalation toxicity studies at lethal doses. The DS proposed classification as STOT SE 3 for respiratory tract irritation based on human poisoning cases after exposure to pyraclostrobin products and supported (in the RCOM) by respiratory irritation seen in animal inhalation studies (at non-lethal doses).

Comments received during consultation

One MSCA commented and did not support the DS proposal. The MSCA pointed to the lack of details in the human poisoning cases, including the composition of the products. Additionally, they considered that there is no evidence of respiratory tract irritation in the animal data.

In response, the DS acknowledged that evaluation of human data is often hindered by limitations in reporting of exposure conditions. The DS emphasised the effects relevant for classification in the human and the animal data and also pointed out that in the renewal assessment report (RAR) the applicant self-classified pyraclostrobin as STOT SE 3 for respiratory tract irritation.

Assessment and comparison with the classification criteria

Human data

The applicant provided information from the clinical incident log at the occupational site where workers were exposed to pyraclostrobin products in combination with other active ingredient containing products. Some cases of slight irritation of the eyes, skin and mouth and/or intoxication (indisposition, headache, ague, fatigue, aching muscles, vomiting, drowsiness, dizziness, adynamic feet, breathing difficulties) were reported. No details of these case reports are available to the DS or RAC.

In a publication by Gergely *et al*. (2007), five independent events of low to moderate severity poisoning by pyraclostrobin products were reported which all occurred in the US state of Iowa, with a total of 33 persons affected. The patients complained about irritation and pain of the upper respiratory tract as well as nausea, headache, eye pain, weakness, dizziness, and chest pain.

In the first event, when working among crops, 27 migrant workers (20 men and 7 women) were accidentally exposed to pyraclostrobin product that was sprayed from a crop-duster plane. Twenty-six of them complained of upper respiratory tract pain or irritation; 20 of

chest pain; 3 of nausea, and 1 patient each had pruritis, skin redness, eye pain, weakness, headache, or exhibited dizziness, respectively. During the RAC-63 meeting, the Industry representative confirmed that the exposure in this event was primarily to pyraclostrobin as it was identified from the samples taken from safety glasses of the workers.

There were three other events of exposure to pyraclostrobin products due to off-target drift from nearby aerial applications. In total, 5 people were affected. They were exposed when riding a motorcycle near a field that was just under treatment or by spray drifting to their home yard. Headache and eye pain partially associated with conjunctivitis and dizziness were reported. No respiratory irritant effects were reported.

In the fifth reported event, a crop-duster pilot was dermally exposed to the spilled liquid pyraclostrobin product when his plane crashed during take-off. The pilot exhibited chemical burns but reported no respiratory symptoms.

Animal data

In the acute inhalation toxicity study (TOX2000-711: 1997), in the low concentration group (0.31 mg/L) with no mortality, 6/10 rats had irregular respiration only during the 4-h exposure and in 2 male rats bloody nose discharge was seen within 1 day after exposure.

In another acute inhalation toxicity study (ASB2008-5020: 2002), visually accelerated respiration was observed in all groups including in the low concentration group (0.52 mg/L) with 10% mortality.

RAC notes that the effects on respiration observed in the animal studies occur at dose levels that are (close to) being lethal but support that the respiratory system is a target organ.

Overall, RAC considers that the first event reported in the Gergely *et al*. (2007) publication with pyraclostrobin products is sufficient for classification. Thus, RAC agrees with the DS and concludes that **pyraclostrobin warrants classification as STOT SE 3; H335: May cause respiratory irritation.**

10.10 Specific target organ toxicity-repeated exposure

Table 77: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Repeated dose oral toxicity, 4 weeks OECD 407 Wistar rats; M/F 5/sex/group GLP-compliant No deviations	Pyraclostrobin (two batches used; purity 94-99 %) 0, 20, 100, 500, 1500 ppm Test substance uptake M: 0, 1.8, 9.0, 42.3, 120 mg/kg bw/day F: 0, 2.0, 9.6, 46.6, 126 mg/kg bw/day	NOAEL: 9/9.6 mg/kg bw/day M/F [100 ppm] LOAEL: 42.3/46.6 mg/kg bw/day M/F [500 ppm] Target organs/tissues: Blood, duodenum, liver, spleen Reported treatment-related effects • Decrease in body weight (BW)/body weight gain (BWG) in	TOX2000-715: 1999

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
	Dietary administration 4 weeks	males (-15% BW; -32% BWG) at 1500 ppm • Decrease in food consumption in males (-17%) and females (-16%) at 1500 ppm • Increased mean relative liver weights in males (+16%) and females (+26%) at 1500 ppm • Increased mean relative spleen weights in males (+31% at 500 ppm, n.s.; +63% at 1500 ppm) and in females (+31% at 500 ppm; +49% at 1500 ppm) • Decrease in red blood cell parameters and haemoglobin concentrations in female rats at 1500 and 500 ppm, suggesting slight anaemia • Reduction in serum cholinesterase activity in females at 500 and 1500 ppm • Increased prothrombin time in males at 500 and 1500 ppm and females at 1500 ppm • Mucosal hyperplasia in the duodenum at 1500 (4 males; 4 females) and 500 ppm (4 males; 2 females) • Hepatocellular hypertrophy in 4 males and 1 female at 1500 ppm • Diminished hepatocellular fat storage at 1500 and 500 ppm in both sexes • Increased extramedullary haematopoiesis in the spleen at 1500 (5 males; 4 females) and 500 ppm (4 males; 5 females)	
Subchronic oral toxicity, 3 months OECD 408 Wistar rats; M/F 10/sex/group GLP-compliant No deviations	Pyraclostrobin (purity 98.5 %) 0, 50, 150, 500, 1000, 1500 ppm Test substance uptake M: 0, 3.5, 10.7, 35, 69, 106 mg/kg bw/day F: 0, 4.2, 12.6, 41, 80, 119 mg/kg bw/day Dietary administration 3 months	NOAEL: 10.7/12.6 mg/kg bw/day M/F [150 ppm] LOAEL: 35/41 mg/kg bw/day M/F [500 ppm] Target organs/tissues: Blood, duodenum, liver, spleen Reported treatment-related effects • Reduced BW/BWG in males at ≥ 500 ppm (-7, -16 and -26% BW and -11, -25 and -42% BWG at 500, 1000 and 1500 ppm, respectively, by end of study) and	TOX2000-717: 1999

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		females only at 1500 ppm (-9% BW; -18% BWG by end of study) • Reduced food consumption at ≥ 500 ppm (stat. significant for most days in males and only for a few days in females) • Decreased absolute liver weight at ≥ 500 ppm in males; increased absolute liver weight in females at 1500 ppm • Anaemic effects, e.g. decrease in haematological parameters (red blood cells and haemoglobin) in females at ≥ 1000 ppm and increase in total bilirubin in males at ≥ 1000 ppm and females at 1500 ppm. • Increased incidence and/or severity of mucosal hyperplasia of the duodenum in males at ≥ 500 ppm and in all 10 females at 1500 ppm. • Increased incidence and severity of centrilobular hepatocyte hypertrophy in males at ≥ 500 ppm and in 4 females at 1500 ppm. • Diminished incidence and/or severity of hepatocellular fat storage (fatty change, diffuse) in both sexes at ≥ 500 ppm. • Increased severity of extramedullary haematopoiesis in the spleen of males at ≥ 1000 ppm. In females at ≥ 1000 ppm increased incidence of this finding was noted. • Increased incidence and severity of sinusoid distension and histiocytosis in the spleen of both sexes at ≥ 1000 ppm.	
Subchronic oral toxicity, 3 months OECD 408 B6C3F1 mouse; M/F 10/sex/group GLP-compliant No deviations	Pyraclostrobin (purity 98.5%) 0, 50, 150, 500, 1000, 1500 ppm <u>Test substance uptake</u> M: 0, 9.2, 30.4, 119, 274, 476 mg/kg bw/day F: 0, 12.9, 40.4, 162, 374, 635 mg/kg bw/day	<u>NOAEL</u>: <9.2 mg/kg bw/day for M [<50 ppm] and 12.9 mg/kg bw/day for F [50 ppm] <u>LOAEL</u>: 9.2 mg/kg bw/day for M [50 ppm] and 40.4 mg/kg bw/day for F [150 ppm] <u>Target organs/tissues</u>: Blood and duodenum <u>Reported treatment-related effects</u>	TOX2000-718: 1998

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
	Dietary administration 3 months	• Reduced BW and BWG in males at ≥50 ppm and in females ≥150 ppm • Changes in haematology, e.g. - Statistically significant decrease in haemoglobin in females at ≥ 1000 ppm and males at 1500 ppm and in haematocrit in males at ≥ 150 ppm - Statistically significant decrease of white blood cell counts in males at ≥ 1000 ppm • Increased incidence and/or severity of mucosal hyperplasia of the duodenum in both sexes at ≥ 500 ppm	
Subchronic oral toxicity, 3 months OECD 409 Beagle dog; M/F 5/sex/group GLP-compliant No deviations	Pyraclostrobin (purity 97.09%) 0, 100, 200, 450 ppm Test substance uptake M: 0, 2.8, 5.8, 12.9 mg/kg bw/day F: 0, 3.0, 6.2, 13.6 mg/kg bw/day Dietary administration 3 months	NOAEL: 5.8/6.2 mg/kg bw/day M/F [200 ppm] LOAEL: 12.9/13.6 mg/kg bw/day M/F [450 ppm] Target organs/tissues: Blood and duodenum Reported treatment-related effects (all at 450 ppm) • Reduced BWG in females (-0.2 kg vs. 1.2 kg in control) • Reduced food consumption in females (~9% difference from control) • Vomitus and diarrhoea in both sexes • Slight decreases in total protein, albumin and globulin in both sexes (likely to be related to diarrhoea) • Increased platelet counts in females • Significant decreases in serum protein and in glucose levels in females • Slight thickening of the duodenal wall in two males and two females • Hypertrophy of the duodenal mucosa in two males and one female	TOX2000-719: 1999
Chronic oral toxicity, 12 months OECD 452	Pyraclostrobin (purity 98.7%)	NOAEL: 5.4 mg/kg bw/day for both M/F [200 ppm]	TOX2000-725: 1999

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Beagle dog; M/F 5/sex/group GLP-compliant No deviations	0, 100, 200, 400 ppm Test substance uptake M: 0, 2.7, 5.4, 10.8 mg/kg bw/day F: 0, 2.7, 5.4, 11.2 mg/kg bw/day Dietary administration 12 months	LOAEL: 10.8/11.2 mg/kg bw/day M/F [400 ppm] Target organs/tissues: Blood Reported treatment-related effects (all at 400 ppm) • Reduced BWG (1.1 kg vs. 2.7 kg in control) and food consumption (-11% compared to control) in females • Vomitus and diarrhoea in both sexes • Decrease in total protein, albumin, globulins and cholesterol in both sexes, most likely attributable to diarrhoea • Increased white blood cell counts, polymorphonuclear neutrophils and lymphocytes in males, indicative of a mild inflammatory reaction • Increased platelet counts in both sexes (n.s. for females). No further concomitant findings or correlations were found to provide interpretation of this effect.	
Chronic oral toxicity, 24 months OECD 452 Wistar rat; M/F 20/sex/group GLP-compliant No deviations	Pyraclostrobin (purity 97.09%) 0, 25, 75, 200 ppm Test substance uptake M: 0, 1.1, 3.4, 9.0 mg/kg bw/day F: 0, 1.5, 4.6, 12.3 mg/kg bw/day Dietary administration 24 months	NOAEL: 3.4/4.6 mg/kg bw/day M/F [75 ppm] LOAEL: 9.0/12.3 mg/kg bw/day M/F [200 ppm] Reported treatment-related effects (all at 200 ppm) • Reduced BW (-10% M; -9% F) and BWG (max. -11% M; -14% F) in both sexes • Decreased alkaline phosphatase activities in both sexes and alanine aminotransferase activity in males Note: Haematological examinations, ophthalmoscopy, urinalysis and histopathology (gross lesion or microscopic examination) did not reveal test substance-related effects.	TOX2000-726: 1999
Carcinogenicity study OECD 451 Wistar rats, M/F 50/sex/group	Pyraclostrobin (purity 97.09%) 0, 25, 75, 200 ppm Test substance uptake	NOAEL: 3.4/4.7 mg/kg bw/day for M/F [75 ppm] LOAEL: 9.2/12.6 mg/kg bw/day M/F [200 ppm]	TOX2000-727: 1999

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
GLP-compliant No deviation	M: 0, 1.2, 3.4, 9.2 mg/kg bw/day F: 0, 1.5, 4.7, 12.6 mg/kg bw/day 24-month oral dietary exposure	Target organs/tissues: Liver Reported treatment-related effects (all at 200 ppm) • Decreased BW (max. -7% M; -14% F) and BWG (max. -10% M; -22% F) in both sexes • Increased incidence of liver cell necrosis in males	
Carcinogenicity study OECD 451 B6C3F1 mice, M/F 50/sex/group GLP-compliant No deviation	Pyraclostrobin (purity 97.09%) 0, 10, 30, 120 ppm (M/F), 180 ppm (F only) Test substance uptake M: 0, 1.4, 4.1, 17.2 mg/kg bw/day F: 0, 1.6, 4.8, 20.5, 32.8 mg/kg bw/day 18-month oral dietary exposure	NOAEL: 4.1/4.8 mg/kg bw/day for M/F [30 ppm] LOAEL: 17.2/20.5 mg/kg bw/day M/F [120 ppm] Reported treatment-related effects • Reduced BW (-13% M; -9.5% F) and BWG (-28% M; -20% F) in both sexes at 120 ppm Note: Blood smear and histopathological investigations did not reveal any test substance-related adverse effect.	TOX2000-728: 1999
Repeated dose dermal toxicity, 4 weeks OECD 410 Wistar rat; M/F 10/sex/group GLP-compliant No deviation	Pyraclostrobin (purity 99%) 0, 40, 100, 250 mg/kg bw/d 6 hours/day; 5 days/week; semiocclusive dressing for 4 weeks	NOAEL: >250 mg/kg bw/day (systemic); NOAEL not established for local effects LOAEL: >250 mg/kg bw/day; no systemic toxicity at maximum tested dose. Reported treatment-related effects ≥ 40 mg/kg bw/d: Signs of local skin irritation	TOX2000-716: 1999
Repeated dose inhalation toxicity, 4 weeks OECD 413 Wistar rat; M/F 10/sex/group GLP-compliant No deviation	Pyraclostrobin (purity 98.7%; aerosol; solid dissolved in acetone) 0, 1, 30, 300 mg/m³ (both negative/conditioned air and vehicle/acetone controls included in study) 6 h/day, 5 day/week for 4 weeks (head-nose only)	NOAEC: 1 mg/m³ LOAEC: 30 mg/m³ Target organs/tissues: Respiratory tract and duodenum Reported treatment-related effects • Mortality (4 males and 3 females) at 300 mg/m³ during days 7 to 24 of exposure period with clinical signs of visually increased respiration, urinous odour and piloerection observed before death • Reduced BWG in males at 300 mg/m³ (-43% compared to vehicle/acetone control) • Slight (n.s.) increases in white blood cells and	ASB2008-5026: 2005

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		polymorphonuclear neutrophils in both sexes at 300 mg/m³, indicative of mild inflammatory process • Dose-related increase in incidence and severity of hyperplasia of the duodenal mucosa and effects in the respiratory tract (e.g. atrophy/necrosis in the olfactory epithelium of the nasal cavity) in both sexes at ≥ 30 mg/m³	
Repeated dose inhalation toxicity, 4 weeks OECD 413 Wistar rat; M/F 10/sex/group GLP-compliant No deviation	Pyraclostrobin (purity 99.02 %; aerosol; solid dissolved in acetone) 0, 3, 10, 30 mg/m³ (only vehicle/acetone control included in study) 6 h/day, 5 day/week for 4 weeks (head-nose only) Control and 30 mg/m³ groups (10/sex/group) were additionally included and evaluated in a 4-week recovery period after exposure.	NOAEC: 3 mg/m³ LOAEC: 10 mg/m³ Target organs/tissues: Blood, duodenum, spleen, respiratory tract Reported treatment-related effects • Decreased BWG (-29%; n.s.) in males at 30 mg/m³ after 4-week treatment • Slight but significant decrease (up to -5%) in haemoglobin concentrations in females at ≥10 mg/m³ • Irritation in the upper respiratory tract (atrophy/ necrosis of olfactory epithelium) at 30 mg/m³ • Increased duodenum (abs./rel.) in both sexes at ≥10 mg/m³ and spleen (abs.) weight in females at 30 mg/m³ without observed histopathological correlates	ASB2015-11604: 2014
Two-generation reproduction toxicity study, OECD 416 Wistar rats; M/F 25/sex/group GLP-compliant Deviation: From day 14 after parturition onwards, food consumption was not determined since the pups themselves began to consume considerable amounts of solid food. This deviation did not alter the validity of the results.	Pyraclostrobin (purity 98.7 %) 0, 25, 75, 300 ppm Daily test substance intake 0, 2.7, 8.2, 32.6 mg/kg bw/day (calculated for the whole study period with all phases, for all groups and both sexes) Dietary exposure F_0: Treatment started 74 days before mating and lasted until after weaning of F_1 pups. F_1: Exposed continuously from their growth into	NOAELs (parental and offspring): ~8.2 mg/kg bw/day [75 ppm] Reported effects (relevant for STOT RE) • Statistically significant increase in mean relative kidney weight in 300 ppm-exposed F_0 and F_1 males – Histopathology did not reveal an increase in any renal lesion neither in the F_0 nor in the F_1 generation, and the absolute kidney weights were not altered. Note: Gross examination at necropsy did not reveal any lesions that might be allocated to treatment.	TOX2000-729: 1999

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
	adulthood until or up to 16 hours before sacrifice (i.e. after weaning of F_2 pups).		
One-generation reproduction toxicity, dose range-finding study Similar to OECD 415 Wistar rats; M/F 10/sex/group GLP-compliant Deviations: - 10 pregnant females examined instead of 20 - F0 generation parental animals were mated from 45 days onwards after the beginning of treatment, whereas it is required in the guideline that dosing is continued for ten weeks prior to the mating period - food consumption of the females during the mating period was not determined Supplementary data	Pyraclostrobin (purity 97.09 %) 0, 200, 400, 600 ppm Test substance intake M: 0, 20.5, 39.9, 59.1 mg/kg bw/day F (three phases) - premating: 0, 21.3, 42.5, 60.4 mg/kg bw/day - gestation: 0, 18.3, 35.0, 53.2 mg/kg bw/day - lactation (PND 1-14): 0, 29.0, 51.9, 80.2 mg/kg bw/day Dietary exposure Treatment started 45 days before mating and lasted until day 21 after parturition (weaning of F_1 pups).	Target organs/tissues: Duodenum Reported effects (relevant for STOT RE) The only gross pathology finding was a thickening of the wall of the duodenum in all males at 600 ppm.	ASB2017-5538: 2002

n.s.: not statistically significant

Table 78: Summary table of human data on STOT RE

Type of data/report	Test substance	Route of exposure and Relevant information about the study (as applicable)	Observations	Reference
N/A				

Table 79: Summary table of other studies relevant for STOT RE

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
N/A				

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

For the identification of potential target organs from repeated exposure to pyraclostrobin, 13 repeated dose toxicity studies, all performed in accordance with or similar to OECD Guidelines, were evaluated. These studies ranged from 28-day repeated dose studies to chronic toxicity/carcinogenicity studies with exposure of up to 2 years. These studies include examination of toxicity in both rodents (rats and mice) and non-rodents (namely dogs) as well as via various routes of exposure (oral, dermal and inhalative).

In rats, repeated oral exposure of up to 90 days to pyraclostrobin was reported to trigger effects in the blood, GI tract (mainly duodenum), liver and spleen. Effects in these tissues/organs were already observed after 28 days of repeated exposure to ≥ 42.3 mg/kg bw/day (500 ppm) pyraclostrobin (TOX2000-715: 1999; see Table

54). Subsequently, in the 90-day repeated oral dose toxicity study (TOX2000-717: 1999), effects in the abovementioned tissues/organs from the 28-day repeated dose toxicity study persisted at the similar dose range of 35-41 mg/kg bw/day (500 ppm). The tables below show the key treatment-related findings from the 90-day repeated oral dose toxicity study.

Table 80: Treatment-related haematology and clinical chemistry findings in the rat 90-day repeated dose toxicity study (TOX2000-717: 1999)

Parameter	Sex	Dietary dose level [ppm]					
		0	50	150	500	1000	1500
White blood cells [G/L]	M	8.41	8.97	8.05	8.92	8.93	9.59
	F	3.90	4.22	4.85	4.69	6.65***	6.55**
Red blood cells [T/L]	M	8.53	8.53	8.79	8.59	8.36	8.22
	F	7.95	7.91	7.95	7.70	7.36***	7.10***
Hemoglobin [mmol/L]	M	9.7	9.5	9.8	9.7	9.5	9.4
	F	9.2	9.3	9.3	9.3	8.7**	8.6***
Reticulocytes [‰]	M	17	17	16	19	24**	33***
	F	14	17	14	13	15	23***
Prothrombin time [s]	M	26.0	26.5	26.4	27.1	28.9***	29.4***
	F	25.6	24.7	25.5	26.1	27.5***	26.3
Total bilirubin [µmol/L]	M	1.69	1.70	1.76	2.20	2.67***	3.29***
	F	2.17	1.93	1.94	1.93	2.69	2.94**
Kruskal-Wallis + Mann-Whitney U-test *p<0.05; **p<0.02;***p<0.002							

Table 81: Selected mean organ weights in the rat 90-day repeated dose toxicity study (TOX2000-717: 1999)

		Males				Females			
Organ	Dose [ppm]	Absolute weight	Δ%	Relative weight [% of b.w.]	Δ%#	Absolute weight	Δ%	Relative weight [% of b.w.]	Δ%#
Liver [g]	0	15.005		3.409		6.772		2.942	
	50	13.956	(-7.0)	3.132	(-8.1)	7.266	(7.3)	3.006	(2.2)
	150	13.182*	(-12.1)	3.111	(-8.7)	7.083	(4.6)	3.015	(2.5)
	500	13.054*	(-13.0)	3.217	(-5.6)	6.931	(2.3)	3.095	(5.2)
	1000	12.322**	(-17.9)	3.331	(-2.3)	7.274	(7.4)	3.34	(13.5)
	1500	11.942**	(-20.4)	3.746	(9.9)	8.253**	(21.9)	3.935	(33.8)
Spleen [mg]	0	0.874		0.199		0.555		0.241	
	50	0.900	(3.0)	0.202	(1.5)	0.625	(12.6)	0.258	(7.1)
	150	0.844	(-3.4)	0.199	(0.0)	0.601	(8.3)	0.255	(5.8)
	500	0.848	(-3.0)	0.210	(5.5)	0.654*	(17.8)	0.293*	(21.6)
	1000	0.946	(8.2)	0.256**	(28.6)	0.740**	(33.3)	0.340**	(41.1)

	1500	1.018*	(16.5)	0.320**	(60.8)	0.878**	(58.2)	0.420**	(74.3)
* p ≤ 0.05; ** p ≤ 0.01 (Kruskal-Wallis and Wilcoxon-test, two sided) # Values may not calculate exactly due to rounding of figures									

Table 82: Treatment-related histopathological findings in 3-month repeated dose toxicity study in rats (TOX2000-717: 1999)

	Males						**Females**					
Dose [ppm]	**0**	**50**	**150**	**500**	**1000**	**1500**	**0**	**50**	**150**	**500**	**1000**	**1500**
Duodenum # examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
- Mucosal hyperplasia	2	1	1	4	5	10	2	1	2	1	1	10
	[1.0]	[1.0]	[1.0]	[1.3]	[1.2]	[1.9]	[1.0]	[1.0]	[1.5]	[1.0]	[2.0]	[1.4]
Liver # examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
- hypertrophy, centrilobular	-	-	-	3	6	10	-	-	-	-	-	4
	-	-	-	[1.0]	[1.2]	[1.8]	-	-	-	-	-	[1.0]
- Fatty change, diffuse	10	8	9	6	2	-	4	7	5	2	1	-
	[2.5]	[2.1]	[2.2]	[1.5]	[1.0]	-	[1.5]	[1.4]	[1.4]	[1.0]	[3.0]	-
Spleen # examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
- Extramedullary hematopoiesis	2	-	3	1	2	3	-	-	3	3	9	9
	[1.0]	-	[1.0]	[1.0]	[1.5]	[1.7]	-	-	[1.7]	[1.7]	[1.3]	[1.8]
- Hemosiderin deposition	10	10	10	10	10	10	10	10	10	10	10	10
	[2.7]	[2.5]	[2.1]	[2.5]	[1.8]	[1.7]	[2.9]	[2.7]	[2.8]	[2.5]	[2.3]	[2.3]
- Sinusoid distension	-	-	-	1	10	8	-	-	-	2	8	10
	-	-	-	[1.0]	[1.4]	[2.0]	-	-	-	[1.0]	[1.1]	[1.7]
- Histiocytosis	-	-	1	3	6	10	-	-	1	2	7	7
			[1.0]	[1.7]	[1.5]	[1.8]	-	-	[1.0]	[1.0]	[1.3]	[1.7]
[ ] average severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The average severity is the sum of the gradings divided by the incidence.												

To summarise these treatment-related findings, haematological and clinical chemistry examinations revealed findings indicative of haemolytic anaemia (e.g. significantly decreased red blood cell counts and haemoglobin concentration in females as well as increase in total bilirubin concentration in both sexes; see corresponding table above) at ≥ 1000 ppm with females having more pronounced effects than males. The changes of liver and spleen weights in both sexes (see corresponding table above) were considered treatment-related since they were corroborated by histopathological findings.

Mild hepatocellular hypertrophy (zone 3, Rappaport) was observed in 3 out of 10 males exposed to 35 mg/kg bw/day (500 ppm) pyraclostrobin. The incidence and severity of this finding increased with dose (6/10 and 10/10 males at 1000 and 1500 ppm, respectively) and males were more susceptible to liver effects as only 4/10 females at the highest dose (1500 ppm) exhibited mild hepatocellular hypertrophy. Diminished incidence of hepatocellular fat storage (fatty change, diffuse) was also seen in both sexes at ≥ 500 ppm. Next, mild mucosal hyperplasia of the duodenum (characterised by increased number of epithelial cells and slightly elongated and broadened villi) was observed in 4/10 males at 500 ppm and also increased with dose (5/10 and 10/10 males at 1000 and 1500 ppm, respectively); all females at 1500 ppm also exhibited mucosal hyperplasia of the duodenum. Lastly, in the spleen, increased severity of extramedullary haematopoiesis was observed in males at ≥ 1000 ppm, and in females at ≥ 1000 ppm increased incidence of this finding was noted. Increased incidence

and severity of sinusoid distension and histiocytosis in the spleen were observed in both sexes at ≥ 1000 ppm.

In the longer-term 2-year chronic toxicity and carcinogenicity studies, the duodenal, spleen or haematological effects related to treatment were no longer observed, but the liver remained affected by pyraclostrobin exposure. Specifically, in the 2-year carcinogenicity study (TOX2000-727: 1999), there was an increased incidence of liver cell necrosis in males exposed to 200 ppm (9.2 mg/kg bw/day) pyraclostrobin (10/50 males vs. 1/50 control male). The liver cell lesion found in 9 males was characterised by predominantly periportal piecemeal necrosis, often associated with small group or extended bridging necrosis. One animal exhibited moderate focal subcapsular necrosis. Among the 10 males with this lesion, 8 died before study termination, but liver cell necrosis was not regarded as the major cause of death in these animals. While the incidence of liver cell necrosis was not increased in male rats exposed to 200 ppm (9.0 mg/kg bw/day) pyraclostrobin in the comparable 2-year chronic toxicity study (TOX2000-726: 1999), only 20 males were examined in this study, so more weight is given to the 2-year carcinogenicity study for evaluating liver effects.

Duodenal effects, such as increased weight and mucosal hyperplasia, were commonly observed in rats not only in 28- and 90-day repeated oral dose toxicity studies but also in 28-day repeated inhalation toxicity studies, suggesting that the duodenum could be a systemic target organ of repeated pyraclostrobin exposure. Specifically, in two 4-week inhalation rat studies of pyraclostrobin (ASB2008-5026: 2005; ASB2015-11604: 2014), increased duodenum weight was observed starting at 10 mg/m^3 followed by hyperplasia of the duodenal mucosa at ≥ 30 mg/m^3. In the studyASB2015-11604: 2014 both males and females exposed to 10 mg/m^3 exhibited an increase in duodenum weight (+15% abs./+11% rel. in males; +21% abs./rel. in females), which increased with the higher concentration of 30 mg/m^3 (+35% abs./33% rel. in males; +40% abs./+34% rel. in females). No histopathological correlates were found in this study, but the other study ASB2008-5026: 2005 reported that at 30 mg/m^3, 5 animals of each sex out of 10 animals per sex examined had mild level of duodenal hyperplasia, and this effect increased in incidence (7 males and 10 females affected) and severity at the highest tested concentration of 300 mg/m^3 (see table below). Duodenum weight was not measured in the study ASB2008-5026: 2005.

Table 83: Grading of duodenal mucosal hyperplasia in the 28-d repeated inhalation rat study (ASB2008-5026: 2005)

	Males [concentration in mg/m^3]					**Females [concentration in mg/m^3]**				
Grade	**0 (N)**	**0 (V)**	**1**	**30**	**300***	**0 (N)**	**0 (V)**	**1**	**30**	**300**
0	10	10	10	5	1	9	9	10	5	0
1				5	4		1		5	1
2					3	1				5
3										3
4										1

N: negative control with conditioned air; V: vehicle control with acetone

The grading of mucosal hyperplasia was assessed by a quantitative mucosal area measurement performed by analysis Doku 3.0. The following gradings were used: grade 0 = duodenal mucosal area up to 6 mm^2; grade 1 = >6-8 mm^2; grade 2 = >8-10 mm^2; grade 3 = >10-12 mm^2; grade 4 = >12 mm^2.

* evaluation of 2 animals in this group was not possible due to advanced autolytic changes

There are some uncertainties regarding the duodenal findings in rats, e.g. lack of temporal concordance from the 2-year studies and question about reproducibility of mucosal hyperplasia in the 2 repeated inhalation studies. It is also worth mentioning that thickening of the duodenal wall was observed in all males exposed to 59.1 mg/kg bw/day (600 ppm) in the 1-generational reproduction study (ASB2017-5538: 2002) but not in males exposed up to 32.6 mg/kg bw/day (300 ppm) in the 2-generational reproduction study (TOX2000-729: 1999). However, aside from the possibility of duodenum being a systemic target organ, effects in the duodenum were also observed in mice and dogs after 90-day repeated oral exposure to pyraclostrobin.

In the 90-day subchronic oral toxicity study in mice (TOX2000-718: 1998), dose-related reductions in body weight and body weight gain were observed in both sexes exposed to pyraclostrobin with males having more

pronounced effects than females (see table below).

Table 84: Mean body weight and body weight gain of mice in the 90-day subchronic oral toxicity study (TOX2000-718: 1998)

Males						
Dose level [ppm]	**0**	**50**	**150**	**500**	**1000**	**1500**
Body weight [g]						
- Day 0	23.5	23.5	23.4	23.8	23.9	23.9
- Day 91	36	33.5	31.9**	29.0**	26.4**	23.8**
Δ% (compared to control) #		-6.9	-11.4	-19.4	-26.7	-33.9
Overall body weight gain [g]	12.6	10.0	8.4**	5.1**	2.5**	-0.1**
Δ% (compared to control) #		-20.6	-33.3	-59.5	-80.2	-100.8
Females						
Dose level [ppm]	**0**	**50**	**150**	**500**	**1000**	**1500**
Body weight [g]						
- Day 0	19.1	19.2	19.1	19.1	19.3	18.9
- Day 91	26.5	26.2	25.8	23.4**	22.1**	20**
Δ% (compared to control) #		-1.1	-2.6	-11.7	-16.6	-24.5
Overall body weight gain [g]	7.5	7.0	6.7	4.3**	2.8**	1.1**
Δ% (compared to control)#		-6.6	-10.7	-42.7	-62.7	-85.3

Values may not calculate exactly due to rounding of mean values

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett's test, two sided)

Changes of haematological parameters were mainly observed in males. The statistically significant decrease of white blood cell counts in males at ≥ 1000 ppm was mainly due to a decrease in the number of lymphocytes as indicated by the differential blood cell count. In females not statistically significant decrease of white blood cell counts was observed, which in part was also due to a decrease in lymphocyte counts (see table below).

Table 85: Treatment-related haematology findings in mice in the 90-day subchronic oral toxicity study (TOX2000-718: 1998)

		Dietary dose level [ppm]					
Parameter	**Sex**	**0**	**50**	**150**	**500**	**1000**	**1500**
White blood cells [G/L]	M	5.92	5.56	5.20	6.36	2.72***	2.67***
	F	6.04	5.15	4.10	3.72	3.25	3.17
Haemoglobin [mmol/L]	M	11.8	11.6	11.6	11.4	11.4	10.6***
	F	11.4	11.5	11.2	11.0	10.9**	10.4**
Haematocrit [l/L]	M	0.572	0.564	0.551*	0.542**	0.543**	0.518**
	F	0.519	0.531	0.517	0.516	0.513	0.495
Kruskal-Wallis + Mann-Whitney U-test *p<0.05; **p<0.02;***p<0.002							

Majority of the findings from the histopathological examination, namely glandular stomach erosion/ulcer, thymic atrophy, decreased lipid vacuoles in the kidneys and presence of apoptotic bodies in follicles of mesenteric lymph nodes in both sexes, decreased diffuse fatty infiltration of hepatocytes in males and decreased lipid in the X-zone of the adrenal cortex in females, were considered to be a consequence of reduction of terminal body weight in the affected animals. The only treatment-related histopathological finding in this 90-day subchronic oral toxicity study in mice is duodenal mucosal hyperplasia (see table below).

Table 86: Treatment-related histopathological duodenal findings in mice in the 90-day subchronic oral toxicity study (TOX2000-718: 1998)

	Males						**Females**					
Dose [ppm]	**0**	**50**	**150**	**500**	**1000**	**1500**	**0**	**50**	**150**	**500**	**1000**	**1500**
Animals in group	10	10	10	10	10	10	10	10	10	10	10	10
Duodenum # examined	10	10	10	10	10	10	10	10	10	10	10	9
- Mucosal hyperplasia	-	-	-	10	10	10	-	-	-	6	10	9
				[2.4]	[2.8]	[3.0]	-	-	-	[2.0]	[2.0]	[2.2]

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

The duodenal mucosal hyperplasia found in mice was detected macroscopically via thickening of the duodenal wall and histopathologically characterised as the intestinal villi being elongated, partly slightly broadened and branched. The severity grading of the hyperplasia ranged from slight to moderate (Grades 2-3). However, similar to rats, the duodenal effects (as well as the effects in the organs) from the 90-day study were no longer observed in the 18-month carcinogenicity study tested up to 17.2 mg/kg bw/day in males and 32.8 mg/kg bw/day in females (TOX2000-728: 1999).

In dogs, only the duodenum was reported to be the target organ of pyraclostrobin treatment in the 3-month repeated dose toxicity study (TOX2000-719: 1999). Specifically in this study, at the highest dose of 13 mg/kg bw/day (450 ppm), slight thickening of the duodenal wall was observed in 2 males and 2 females, and hypertrophy of the duodenal mucosa was observed 2 males and 1 female. The mucosal hypertrophy was characterised by an increased ratio of cytoplasm to the nuclei in the villi (slightly elongated; well-preserved up to their tips) and a hyperplastic aspect in the epithelial cells. However, mucosal hypertrophy of the duodenum was no longer observed in the 12-month repeated oral dose toxicity study tested up to 11 mg/kg bw/day and histopathology did not reveal any treatment-related findings or a target organ in this study (TOX2000-725: 1999).

In conclusion, liver and duodenum can be considered as specific target organs for STOT RE classification of pyraclostrobin. Effects in the blood and spleen were also observed in rats from repeated exposure to pyraclostrobin but the effects were more pronounced at doses higher than the liver and duodenal effects. Based on the 90-day repeated oral dose toxicity study in rats, effects in liver (increased weight and mild degree of hepatocyte hypertrophy) and duodenum (mild degree of mucosal hyperplasia) were observed starting at 35 mg/kg bw/day, a dose that would fall within the Guidance Value Range for Cat. 2 (10-100 mg/kg bw) classification. However, in this study, the severity of observed effects would not be considered as ‘significant/severe’ effects for classification purpose and there is a lack of temporal concordance for the duodenal effects (i.e. not observed in longer-term studies), thereby raising some uncertainty about the duodenum being a target organ of repeated pyraclostrobin exposure.

STOT RE classification for the liver effects is justified by the observation that the increasing severity of effects with longer exposure. As described earlier, in the 2-year carcinogenicity study, liver cell necrosis was found in 10 out of 50 males exposed to 9.2 mg/kg bw/day pyraclostrobin and 8 of these 10 males died before study termination. This suggests that centrilobular hepatocyte hypertrophy after subchronic exposure could exacerbate and result in more severe effects of significant toxicity such as liver cell necrosis if exposure is prolonged. Furthermore, extrapolation of this dose from the 2-year study would result in an effective dose for a 90-day exposure of 74.6 mg/kg bw/day, which also falls within the Guidance Value Range for Cat. 2 classification.

As for the duodenum, STOT RE classification is also justified by the observation that duodenal mucosal hyperplasia was consistently observed in multiple species (rats, mice and dogs) after 90-d repeated oral exposure as well as in rats after 28-d repeated inhalation exposure, suggesting that duodenum could be a systemic target organ for humans as well.

Table 87: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
TOX2000-727: 1999; oral carcinogenicity rat study	9.2 mg/kg bw/day	2 years	74.6 mg/kg bw/day	Yes for STOT RE, Cat. 2
TOX2000-716: 1999; repeated dermal toxicity rat study	>250 mg/kg bw/day	28 days	>83.3 mg/kg bw/day	No as no systemic toxicity was observed at the highest tested dose.
ASB2008-5026: 2005 and ASB2015-11604: 2014; repeated inhalation toxicity rat studies	30 mg/m^3 (0.03 mg/L)	28 days	0.01 mg/L	Yes for STOT RE, Cat. 1 but see Section 10.12.2 for justification of proposing STOT RE, Cat. 2

10.10.2 Comparison with the CLP criteria

Table 88: Comparison with the CLP criteria for STOT RE

Toxicological result (pyraclostrobin)	CLP criteria
90-d repeated oral dose rat study: Effective dose of 35 mg/kg bw/day (liver hypertrophy and mucosal hypertrophy of the duodenum; supported by [1] liver cell necrosis observed in males exposed to 9.2 mg/kg bw/day for 24 months, corresponding to a dose of 74.6 mg/kg bw/day for a 90-d exposure and [2] mucosal hypertrophy of the duodenum observed in 90-d repeated oral dose studies in mouse starting at 119 mg/kg bw/day and dog starting at 13 mg/kg bw/day)	Oral (rat) – 90-d repeated-dose Cat. 2 (H373): $10 < C \leq 100$ mg/kg bw/day Cat. 1 (H372): $C \leq 10$ mg/kg bw/day
28-d repeated dermal rat study: Effective dose of > 250 mg/kg bw/day for M/F (no systemic toxicity observed) Extrapolated to 90-d exposure: > 83.3 mg/kg bw/day	Dermal (rat) – 90-d repeated-dose Cat. 2 (H373): $20 < C \leq 200$ mg/kg bw/day Cat. 1 (H372): $C \leq 20$ mg/kg bw/day
28-day repeated inhalation rat study: Effective concentration of 0.03 mg/L (6 h/day; hyperplasia of the duodenal mucosa) Extrapolated to 90-d exposure: 0.01 mg/L	Inhalative (rat) – 90-d repeated-dose (mist) Cat. 2 (H373): $0.02 < C \leq 2$ mg/L/6 h/day* Cat. 1 (H372): $C \leq 0.02$ mg/L/6 h/day

* Proposal made based on expert judgment of severity or lack of observed effects

There are no relevant epidemiological or human evidence for STOT RE evaluation, so the classification for STOT RE is solely based on experimental animal data. Repeated oral toxicity studies in rats both revealed liver and duodenal effects within the Guidance Value Range for STOT RE, Category 2 classification. Extrapolation of the effective dose for significant liver damage (necrosis) in the 2-year rat carcinogenicity study to 90-day exposure also yields a value that falls within the Category 2 classification range.

As for the repeated inhalation studies, the effective concentration for increased duodenum weight and hyperplasia of the duodenal mucosa of 30 mg/m^3 or 0.03 mg/L from the 28-d exposure study (extrapolated to 0.01 mg/L to account for the 90-d exposure; see extrapolation table in Section 10.12.1 above) would be in the range of Category 1 classification. However, given the low severity of this finding at this concentration and

considering the effective dose (35 mg/kg bw/day) for the oral route, STOT RE Category 2 classification would be more appropriate.

10.10.3 Conclusion on classification and labelling for STOT RE

In view of the weight of evidence (i.e. increasing severity of liver effects in rats with prolonged exposure and consistent observation of duodenal mucosal hyperplasia after 90-day repeated exposure in 3 species and also after 28-day repeated inhalation exposure in rats), classification as STOT RE 2, "May cause damage to organs (liver and gastrointestinal tract) through prolonged or repeated exposure", H373 is warranted for pyraclostrobin.

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

Summary of the Dossier Submitter's proposal

Several repeated dose toxicity studies covering three species and three routes of exposure are summarised in the CLH report for pyraclostrobin:

- 4 sub-acute studies in rats – 1 each via oral & dermal routes and 2 via inhalation route,
- 3 sub-chronic oral studies – 1 each in rats, mice, and dogs,
- 2 chronic oral studies – 1 each in rats and in dogs,
- 2 carcinogenicity studies – 1 each in rats and in mice,
- 1 one-generation study in rats; and
- 1 two-generation study in rats.

The DS proposed STOT RE 2; H373 (liver and gastrointestinal tract) for pyraclostrobin based on increasing severity of liver effects in rats with prolonged exposure, and consistent observation of duodenal mucosal hyperplasia after sub-chronic repeated exposure in three species and also after sub-acute inhalation exposure in rats.

Blood and spleen were also identified as target organs in the studies, but the DS did not propose classification as the effects in these organs were more pronounced only at doses higher than those resulting in the liver and duodenal effects in rats.

Comments received during consultation

One MSCA and one Company/manufacturer commented but did not state their position on the DS proposal for STOT RE 2 (liver and gastrointestinal tract).

The MSCA questioned why the effects on respiratory tract (atrophy/necrosis of olfactory epithelium at 30 mg/m^3) seen in the 2 sub-acute inhalation studies were not taken into consideration for classification. The DS responded that these effects are instead considered to be local irritation and were used as supporting evidence for the proposed STOT SE 3 classification.

The Company/manufacturer supported the DS view that the duodenal hypertrophy observed at 30 mg/m^3 in one sub-acute inhalation study does not justify STOT RE 1 classification as

the severity was low and the effect was not reproducible in the other sub-acute inhalation study.

Assessment and comparison with the classification criteria

In a sub-acute oral study (OECD TG 407, GLP-compliant and no deviations), pyraclostrobin (purity: 94 – 99%) was administered via diet to Wistar rats (5/sex/group) at (M/F) 1.8/2, 9/9.6, 42.3/46.6 and 120/126 mg/kg bw/d (TOX2000-715: 1999). The effects seen are described below.

Liver: ↑ rel. liver weights (M: +15%, F: +26%) and hepatocellular hypertrophy (4 M, 1 F) at high dose. ↓ hepatocellular fat storage (M & F) at mid-high and high dose.

Blood: ↓ red blood cells (RBC) and haemoglobin in females at mid-high and high dose; ↑ prothrombin time in males (mid-high and high dose) and females (high dose); ↑ extramedullary haematopoiesis in spleen at mid-high (4 M, 5 F) and high dose (5 M, 4 F), and ↑ rel. spleen weights in males (+33% at mid-high (not statistically significant) and +66% at high dose) and in females (+32% at mid-high and +48% at high dose).

Duodenum: Mucosal hyperplasia in the duodenum at mid-high (4 M, 2 F) and high dose (4 M, 4 F).

***Table**: Haematological findings in the sub-acute oral study (TOX2000-715: 1999) (adapted from Table B.6.3-3 in RAR Vol. 3CA - B.6)*

Parameter	**Sex**	**Dose mg/kg bw/d (M/F)**				
		0	1.8/2	9/9.6	42.3/46.6	120/126
Red blood cells (T/L)	m	8.47	8.12	8.14	8.23	8.22
	f	8.12	7.88	8.11	7.59**	7.35**
Haemoglobin (mmol/L)	m	9.6	9.4	9.6	9.3	8.9
	f	9.5	9.3	9.4	8.8**	8.8**
Prothrombin time (s)	m	28.0	28.2	26.9	28.9**	30.2**
	f	24.9	24.6	24.7	25.7	27.8*

Kruskal-Wallis + Mann-Whitney U-test *p<0.05; **p<0.02

Support for STOT RE classification: The equivalent guidance values for a 28-d oral study are ≤ 30 mg/kg bw/d for Category 1 and ≤ 300 mg/kg bw/d for Category 2. The adverse effects in this study were observed at mid-high (M/F: 42.3/46.6 mg/kg bw/d) and/or high dose (M/F: 120/126 mg/kg bw/d) that correspond to the equivalent guidance value for Category 2.

RAC considers the observed liver effects (↑ rel. weights, ↑ hepatocellular hypertrophy and ↓ hepatocellular fat storage) as adaptive responses.

Effects indicative of haemolytic anaemia were observed in females. However, the changes in blood parameters were < 10% compared to controls. The increase in spleen weight may be correlated to increase in extramedullary haematopoiesis in spleen. However, in the

absence of any major changes in haematological parameters, RAC considers that these effects do not merit classification.

The duodenal mucosal hyperplasia is of concern since many animals were already affected at the mid-high dose.

In a sub-acute inhalation study (similar to OECD TG 413, GLP-compliant and no deviations), pyraclostrobin (purity: 98.7%; aerosol – solid dissolved in acetone) was administered (6 h/d, nose-only) to Wistar rats (10/sex/group) at 1, 30 and 300 mg/m^3 (= 0.001, 0.03 and 0.3 mg/L) (ASB2008-5026: 2005).

At 0.3 mg/L, 4 males & 3 females died during days 7 to 24 of exposure period with clinical signs of visually increased respiration, urinous odour and piloerection observed before death. At 0.03 mg/L, the body weight gain of males was reduced by 43%.

Respiratory tract: atrophy/necrosis (minimal to moderate severity) in olfactory epithelium of nasal cavity at 0.03 and 0.3 mg/L.

Liver: No adverse effects reported.

Blood: The DS reported a slight ↑ in white blood cells (WBC) and polymorphonuclear neutrophils (M & F) at 0.3 mg/L. RAC notes that these changes were not statistically significant (Table B.6.3-26 of RAR Vol. 3CA - B.6).

Duodenum: Dose-related ↑ in incidence and severity of duodenal mucosal hyperplasia (M & F) at 0.03 and 0.3 mg/L.

Table: Grading of duodenal mucosal hyperplasia in the sub-acute inhalation study (ASB2008-5026: 2005) (adapted from Table 83 in the CLH report)

	Males [concentration in mg/L]					**Females [concentration in mg/L]**				
Grade	**0 (N)**	**0 (V)**	**0.001**	**0.03**	**0.3***	**0 (N)**	**0 (V)**	**0.001**	**0.03**	**0.3**
0	10	10	10	5	1	9	9	10	5	0
1				5	4		1		5	1
2					3	1				5
3										3
4										1
Average severity grading				[1.0]	[1.3]	[2.0]	[1.0]		[1.0]	[2.4]

N: negative control with conditioned air; V: vehicle control with acetone
The grading of mucosal hyperplasia was assessed by a quantitative mucosal area measurement performed by analysis Doku 3.0. The following gradings were used: grade 0 = duodenal mucosal area up to 6 mm^2; grade 1 = >6-8 mm^2; grade 2 = >8-10 mm^2; grade 3 = >10-12 mm^2; grade 4 = >12 mm^2.
* evaluation of 2 animals in this group was not possible due to advanced autolytic changes

Support for STOT RE classification: The equivalent guidance value for a 28-day inhalation study is ≤ 0.06 mg/L/6h/d for Category 1 and ≤ 0.6 mg/L/6h/d for Category 2.

The duodenal mucosal hyperplasia in this study is of concern. The effects seem more pronounced in females and 1 female showed highest severity grade in the high dose group.

However, it should be noted that the evaluation of 2 males in the high dose group was not possible due to autolytic changes.

The effect on olfactory epithelium is also of concern since atrophy/necrosis was observed at mid- and high dose groups.

In another sub-acute inhalation study (similar to OECD TG 413, GLP-compliant and no deviations), pyraclostrobin (purity: 99.02%; aerosol – solid dissolved in acetone) was administered (6 h/d, nose-only) to Wistar rats (10/sex/group) at 3, 10 and 30 mg/m^3 (= 0.003, 0.01 and 0.03 mg/L) (ASB2015-11604: 2014).

At 0.03 mg/L, the body weight gain of males was reduced by 29% (not statistically significant).

Respiratory tract: atrophy/necrosis (minimal to slight severity) in olfactory epithelium of nasal cavity at 0.03 mg/L.

Liver: No adverse effects reported.

Blood: Slight but statistically significant ↓ (up to -5%) in haemoglobin in females at 0.01 and 0.03 mg/L. ↑ abs. weight of spleen in females at 0.03 mg/L but without histopathological changes.

Duodenum: ↑ weight of duodenum in males (abs. up to 35%; rel. up to 33%) and females (abs. up to 40%; rel. up to 34%) at 0.01 and 0.03 mg/L but without histopathological changes (Tables B.6.3 - 39 & 40 & 40 of the RAR Vol. 3CA - B.6).

Support for STOT RE classification: The equivalent guidance value for a 28-day inhalation study is ≤ 0.06 mg/L/6h/d for Category 1.

The effects on blood were minor and the increase in spleen weights were not corroborated by histopathological changes. Therefore, these effects do not merit classification.

The effects on duodenum (↑ weights by >30%) and olfactory epithelium (atrophy/necrosis) are of concern and are consistent with the previous study.

In a sub-acute dermal study (OECD TG 410, GLP-compliant and no deviations), pyraclostrobin (purity: 99%) was administered (6 h/d, semi-occlusive dressing) to Wistar rats (10/sex/group) at 40, 100 and 250 mg/kg bw/d (TOX2000-716: 1999).

No adverse effects on liver, blood or duodenum were reported in this study.

Dose-related signs of local irritation were observed at ≥ 40 mg/kg bw/d. No systemic toxicity was observed at the highest dose.

In a sub-chronic oral study (OECD TG 408, GLP-compliant and no deviations), pyraclostrobin (purity: 98.5%) was administered via diet to Wistar rats (10/sex/group) at (M/F) 3.5/4.2, 10.7/12.6, 35/41, 69/80 and 106/119 mg/kg bw/d (TOX2000-717: 1999).

Liver: ↓ abs. weight in males (-12 to -20%) at ≥ 10.7 mg/kg and ↑ abs. weight in females (+22%) at 119 mg/kg. ↑ incidence and severity of centrilobular hepatocyte hypertrophy. Diminished incidence and/or severity of hepatocellular fat storage (fatty change, diffuse) at ≥ 35/41 mg/kg (M & F).

Blood: ↓ RBC and haemoglobin in females at ≥ 80 mg/kg and ↑ in reticulocytes and in total bilirubin in males (≥ 69 mg/kg) and females (119 mg/kg). ↑ prothrombin time in males (≥ 69 mg/kg).

Spleen: ↑ abs. (+17% at 106 mg/kg) and rel. (+29% at 69 mg/kg and +61% at 106 mg/kg) weight of spleen in males. ↑ abs. (+18 to +58%) and rel. (+22 to +74%) weight of spleen in females at ≥ 41 mg/kg. ↑ severity (M) or incidence (F) of extramedullary haematopoiesis in spleen at ≥ 69/80 mg/kg. ↑ incidence and severity of sinusoid distension and histiocytosis in the spleen (M & F) at ≥ 69/80 mg/kg.

Duodenum: ↑ incidence and/or severity of duodenal mucosal hyperplasia (characterised by increased number of epithelial cells and slightly elongated and broadened villi) in males at ≥ 35 mg/kg and females at 119 mg/kg.

***Table**: Treatment-related haematology findings in the sub-chronic oral study in rats (TOX2000-717: 1999) (adapted from Table 80 in the CLH report)*

Parameter	Sex	Dose in mg/kg bw/d (M/F)					
		0	**3.5/4.2**	**10.7/12.6**	**35/41**	**69/80**	**106/119**
White blood cells [G/L]	M	8.41	8.97	8.05	8.92	8.93	9.59
	F	3.90	4.22	4.85	4.69	6.65***	6.55**
Red blood cells [T/L]	M	8.53	8.53	8.79	8.59	8.36	8.22
	F	7.95	7.91	7.95	7.70	7.36***	7.10***
Haemoglobin [mmol/L]	M	9.7	9.5	9.8	9.7	9.5	9.4
	F	9.2	9.3	9.3	9.3	8.7**	8.6***
Reticulocytes [‰]	M	17	17	16	19	24**	33***
	F	14	17	14	13	15	23***
Prothrombin time [s]	M	26.0	26.5	26.4	27.1	28.9***	29.4***
	F	25.6	24.7	25.5	26.1	27.5***	26.3
Total bilirubin [µmol/L]	M	1.69	1.70	1.76	2.20	2.67***	3.29***
	F	2.17	1.93	1.94	1.93	2.69	2.94**
Kruskal-Wallis + Mann-Whitney U-test *p<0.05; **p<0.02;***p<0.002							

***Table**: Treatment-related histopathological findings in the sub-chronic oral study in rats (TOX2000-717: 1999) (adapted from Table 82 in the CLH report)*

	Males						**Females**					
Dose (mg/kg bw/d)	**0**	**3.5**	**10.7**	**35**	**69**	**106**	**0**	**4.2**	**12.6**	**41**	**80**	**119**
Duodenum # examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
- Mucosal hyperplasia	2	1	1	4	5	10	2	1	2	1	1	10
	[1.0]	[1.0]	[1.0]	[1.3]	[1.2]	[1.9]	[1.0]	[1.0]	[1.5]	[1.0]	[2.0]	[1.4]
Liver # examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
- hypertrophy, centrilobular	-	-	-	3	6	10	-	-	-	-	-	4
	-	-	-	[1.0]	[1.2]	[1.8]	-	-	-	-	-	[1.0]

- Fatty change, diffuse	10	8	9	6	2	-	4	7	5	2	1	-
	[2.5]	[2.1]	[2.2]	[1.5]	[1.0]	-	[1.5]	[1.4]	[1.4]	[1.0]	[3.0]	-
Spleen # examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
- Extramedullary haematopoiesis	2	-	3	1	2	3	-	-	3	3	9	9
	[1.0]	-	[1.0]	[1.0]	[1.5]	[1.7]	-	-	[1.7]	[1.7]	[1.3]	[1.8]
- Haemosiderin deposition	10	10	10	10	10	10	10	10	10	10	10	10
	[2.7]	[2.5]	[2.1]	[2.5]	[1.8]	[1.7]	[2.9]	[2.7]	[2.8]	[2.5]	[2.3]	[2.3]
- Sinusoid distension	-	-	-	1	10	8	-	-	-	2	8	10
	-	-	-	[1.0]	[1.4]	[2.0]	-	-	-	[1.0]	[1.1]	[1.7]
- Histiocytosis	-	-	1	3	6	10	-	-	1	2	7	7
			[1.0]	[1.7]	[1.5]	[1.8]	-	-	[1.0]	[1.0]	[1.3]	[1.7]

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

Support for STOT RE classification: The guidance value for a 90-d oral study is > 10 and ≤ 100 mg/kg bw/d for Category 2.

The changes in liver weights were not consistent between sexes (abs. weight increased in males while it decreased in females). However, histopathological findings in liver up to moderate severity were observed.

Some of the changes in blood were small (the decrease in RBC and haemoglobin, observed only in females, was <10%; the increase in prothrombin time was up to 11%) and some changes were marked but observed only in males (increase in reticulocytes was 41% and total bilirubin was 58%). These changes are indicative of haemolytic anaemia. The changes in spleen weight were corroborated by histopathological findings in spleen. However, since there were no other serious effects along with haemolytic anaemia, RAC considers that the changes in blood do not merit classification.

Histopathological effects in duodenum are of concern as they were observed in males already at a low dose of 35 mg/kg.

In another sub-chronic oral study (OECD TG 408, GLP-compliant and no deviations), pyraclostrobin (purity: 98.5%) was administered via diet to B6C3F1 mice (10/sex/group) at (M/F) 9.2/12.9, 30.4/40.4, 119/162, 274/374 and 476/635 mg/kg bw/d (TOX2000-718: 1998).

Liver: No adverse effects reported.

Blood: ↓ WBC in males at ≥ 274 mg/kg. ↓ haematocrit in males at ≥ 30.4 mg/kg (only -4% at 30.4 mg/kg). ↓ haemoglobin in females at ≥ 374 mg/kg. (Table 85 of the CLH report)

Duodenum:↑ incidence and/or severity (minimal to moderate grade) of duodenal mucosal hyperplasia (characterised by intestinal villi being elongated, partly slightly broadened and branched) at ≥ 119/162 mg/kg. (Table 86 of the CLH report)

Support for STOT RE classification: The guidance value for a 90-d oral study is > 10 and ≤ 100 mg/kg bw/d for Category 2. No adverse effects were observed at 30.4/40.4 mg/kg but at the next dose level of 119/162 mg/kg histopathological findings up to moderate severity were observed in duodenum.

In another sub-chronic oral study (OECD TG 409, GLP-compliant and no deviations), pyraclostrobin (purity: 97.09%) was administered via diet to Beagle dogs (5/sex/group) at (M/F) 2.8/3, 5.8/6.2 and 12.9/13.6 mg/kg bw/d (TOX2000-719: 1999).

Liver: No adverse effects reported.

Blood: The only effect indicative of haemolytic anaemia was increased platelet counts (information on magnitude not available) in females at 13.6 mg/kg.

Duodenum: Slight thickening of the duodenal wall (2 M & 2 F) and duodenal mucosal hypertrophy (2 M & 1 F) at 12.9/13.6 mg/kg.

Support for STOT RE classification: The guidance value for a 90-d oral study is > 10 and ≤ 100 mg/kg bw/d for Category 2. No (statistically significant and/or severe) adverse effects were observed in liver or blood. Gross- and histopathological findings were observed in the duodenum.

In a chronic oral study (OECD TG 452, GLP-compliant and no deviations), pyraclostrobin (purity: 98.7%) was administered for 12 months via diet to Beagle dogs (5/sex/group) at (M/F) 2.7/2.7, 5.4/5.4 and 10.8/11.2 mg/kg bw/d (TOX2000-725: 1999).

At the high dose, vomitus was observed during the first week of administration (3 M, 4 F) whereas diarrhoea occurred in all animals during the entire administration period; at the end of the study, there were no statistically significant changes in food consumption or body weights.

Liver: No adverse effects reported.

Blood: The only effect indicative of haemolytic anaemia was increased platelet counts in males (up to 37%) and in females (up to 30 %, not statistically significant) at 10.8/11.2 mg/kg.

Duodenum: No adverse effects reported.

Support for STOT RE classification: The equivalent guidance value for a 12-month oral study is > 2.5 and ≤ 25 mg/kg bw/d for Category 2. No adverse effects on liver or duodenum and no significant adverse effects on blood were reported in this study.

In a chronic oral study (OECD TG 452, GLP-compliant and no deviations), pyraclostrobin (purity: 97.09%) was administered for 24 months via diet to Wistar rats (20/sex/group) at (M/F) 1.1/1.5, 3.4/4.6 and 9/12.3 mg/kg bw/d (TOX2000-726: 1999).

No adverse effects on liver, blood or duodenum were reported in this study.

There was no test substance-related increase in mortality or clinical signs of toxicity in this study. There were no statistically significant changes in food consumption or body weights.

In the carcinogenicity study in rats (TOX2000-727: 1999), no adverse effects on blood or duodenum were reported. An increased incidence in liver cell necrosis was observed in 10 out of 50 males (vs. 1/50 controls) of the high dose group (9.2 mg/kg bw/d; the equivalent guidance value for a 24-month oral study is > 1.25 and ≤ 12.5 mg/kg bw/d for Category 2).

The DS described the liver findings in the CLH report as follows: the liver cell lesion found in 9 males was characterised by predominantly periportal piecemeal necrosis, often associated with small group or extended bridging necrosis. One animal exhibited moderate focal subcapsular necrosis. Among the 10 males with this lesion, 8 died before study termination, but liver cell necrosis was not regarded as the major cause of death in these animals.

The liver cell necrosis observed in 20% of the high dose males in this study is of concern.

There was no test substance-related increase in mortality or clinical signs of toxicity in this study. Survival rate and other details were lacking. At the high dose, the changes in body weight gain were statistically significant in females (-21.7%) but not in males (-4.9%).

In the carcinogenicity study in mice (TOX2000-728: 1999) and the two-generation reproduction toxicity study in rats (TOX2000-729: 1999), no adverse effects on liver, blood or duodenum were reported.

In the carcinogenicity study in mice, there was no test substance-related increase in mortality and no clinical signs of toxicity were observed. Survival rate and other details are lacking. Reduced body weight (M: -13%; F: -9,5%) and body weight gain (M: -28% M; F: -20%) were observed at 17.2/20.5 mg/kg bw/d.

In the two-generation study in rats, there were no statistically significant changes in food consumption or body weights at the high dose.

In the one-generation reproduction toxicity study in rats (ASB2017-5538: 2002), no adverse effects on liver were reported. Slight indications of anaemia were observed in males and females (< 5% decrease in haemoglobin; Table B.6.6-3 in RAR Vol. 3CA - B.6). In gross pathology investigations, thickening of the duodenal wall was observed in all 10 high dose males (59.1 mg/kg bw/d. The guidance value for an 80-d oral study (approx. exposure period of males in this study) is > 11.25 and ≤ 112.5 mg/kg bw/d for Category 2.

Overall, effects on blood (and/or spleen) indicative of slight haemolytic anaemia were observed in sub-acute oral & inhalation studies in rats; in sub-chronic studies in rats, mice (only above the guidance value for Category 2) and dogs (also in the chronic study); and in the one-generation study in rats. Considering that the effects on blood were not statistically

significant and/or severe, RAC agrees with the DS conclusion that these do not warrant classification.

Severe histopathological changes in liver (necrosis) were reported in the carcinogenicity study in rats. Changes in liver weights corroborated by histopathology (hypertrophy and fat storage) were also observed in sub-acute and sub-chronic oral studies in rats. These effects were observed at doses within the (equivalent) guidance values for Category 2. Therefore, RAC agrees with the DS conclusion that the effects on liver with pyraclostrobin warrant classification.

Effects on the duodenum (changes in weight and/or histopathology) were observed in sub-acute oral and inhalation studies in rats, and sub-chronic studies in rats, mice (at just above the guidance value for Category 2 in male mice) and dogs at doses within the (equivalent) guidance values for Category 2. Gross pathological changes (thickening of the duodenal wall) were also observed in sub-chronic study in dogs and in the one-generation study in rats at doses within the (equivalent) guidance values for Category 2. Since the effects were consistently observed (in short- and long-term studies; in 3 species; via oral and inhalation routes), RAC agrees with the DS and concludes that the effects on duodenum with pyraclostrobin warrant classification.

Moreover, effects on the olfactory epithelium of the nasal cavity (atrophy/necrosis) were observed in both the sub-acute inhalation studies with the more severe effects occurring at (equivalent) guidance values for Category 2. Therefore, RAC concludes that also the effects on nasal cavity with pyraclostrobin warrant classification.

Overall, RAC concludes that **pyraclostrobin warrants classification as STOT RE 2; H373 (liver, gastrointestinal tract, nasal cavity).**

10.11 Aspiration hazard

Table 89: Summary table of evidence for aspiration hazard

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
N/A				

10.11.1 Short summary and overall relevance of the provided information on aspiration hazard

No data on aspiration hazard of pyraclostrobin were available for evaluation.

10.11.2 Comparison with the CLP criteria

Comparison with CLP criteria for aspiration hazard is not applicable for pyraclostrobin, which is a solid substance without any physicochemical properties known to pose aspiration toxicity hazard.

10.11.3 Conclusion on classification and labelling for aspiration hazard

Hazard class not applicable.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

All the information on ready biodegradability are taken from the RAR and list of endpoints for pyraclostrobin, January 2020.

Table 90: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
OECD 301 F	*Ready biodegradability* Pyraclostrobin has to be considered as "not readily biodegradable".	Reliable	1999/10655: Reuschenbach, 1999
OECD 111 EPA 161-1	*Hydrolytic degradation of the active substance and metabolites > 10%* pH 5 and 7 at 25°C: stable pH 9 at 25°C: very slow degradation	Reliable	1999/10060: Scharf, 1999
OECD 309	*Aerobic mineralisation in surface water* pH water phase /sed ($CaCl_2$): 7.84 / 7.1 pyraclostrobin: DT_{50}/ DT_{90} whole sys. (SFO): (50 µg/L): 28.3/94.1 days (10 µg/L): 26.4/87.7 days DT_{50}/ DT_{90} water (SFO) (pelagic test).: (50 µg/L): 410.4/1363 days (10 µg/L): 458.1/1522 days Metabolite BF 500-3 (applied as impurity in the chlorophenyl-label treated vessels) (SFO, parent DFOP): DT_{50}/ DT_{90} water (pelagic test).: (50 µg/L): 28.6/94.8 days (10 µg/L): 10.7/35.6 days Metabolite BF 500-5 (max in total system of pelagic test 5.6 - 10.9%) (SFO, parent DFOP): DT_{50}/ DT_{90} whole system: (50 µg/L): 103.4/343.5 days	Reliable	2013/1002741: Ebert and Possienke, 2013

Method	Results	Remarks	Reference
	(10 µg/L): no adequate fit could be achieved Mineralisation after 63 d (Pelagic / suspended sediment): (50 µg/L): max. 0.8% /3.4% (10 µg/L): max. 1.0% / 4.7 % Non-extractable residues (suspended sediment test): 50 µg/L: max. 50.8 % 10 µg/L: max. 57.5 %		
BBA Guideline Part IV; 5-1 and US-EPA, Subdivision N, 162-4 (dark study) Deviation from OECD 308 (irradiated study) FOCUS Kinetic Report SANCO/10058/2005 ver. 2.0	*Degradation in water/sediment system – dark* System A (c)[3], (t)[3] (pH water / sed: 8.42 / 7.1) SFO or DFOP: $DegT_{50}$/ $DegT_{90}$ whole system: (c): 23.3/77.4 d (t): 26.8 /89.1 d $DissT_{50}$/ $DissT_{90}$ water phase: (c): 8. 67[1](2.13)[4]/28.8 d (t): 5.27 [1] (2.30)[4] / 17.5 $DissT_{50}$/ $DissT_{90}$ sediment: (c): 25.7 / 85.5 d (t): 29.8 / 98.9 d System B (c)[3], (t)[3] (pH water / sed: 8.09 / 7.3) DFOP $DegT_{50}$/ $DegT_{90}$ whole system: (c), (t): n.c.[2] $DissT_{50}$/ $DissT_{90}$ water phase: (c): 1.86[1](0.636)[4]/6.19 (t): 1.89 [1] (0.443)[4] / 6.28 $DissT_{50}$/ $DissT_{90}$ sediment: (c), (t): n.c.[2] Geometric mean at 20°C: $DegT_{50}$/ $DegT_{90}$ whole system: 25.0/83.0 $DissT_{50}$/ $DissT_{90}$ water phase: 3.6/11.8	Reliable	1999/11241: Staudenmaier, 1999 (dark study) 2012/1165029: Wiedemann, 2013 (Kinetic evaluation of study Staudenmaier)

Method	Results	Remarks	Reference
	$DissT_{50}$/ $DissT_{90}$ sediment: 27.7 /92.0		
Deviation from OECD 308 (irradiated study) FOCUS Kinetic Report SANCO/10058/2005 ver. 2.0	*Degradation in water/sediment system – irradiated* System Kellmetschweiher (pH water / sed: 8.58 /7.5): $DegT_{50}$/ $DegT_{90}$ whole system: 7.22 /23.98[5] $DissT_{50}$/ $DissT_{90}$ water phase: 4.47 / 14.84[4] $DissT_{50}$/ $DissT_{90}$ sediment: 5.93/19.69[5] System Berghäuser Altrhein (pH water / sed: 7.35 /7.4): DT_{50}/ DT_{90} whole system: 15.4 (13.5)[4] / 54.4 DT_{50}/ DT_{90} water phase: 1.2[1] (0.3)[4] / 3.9 DT_{50}/ DT_{90} sediment: 20.1 / 66.7	Study results are only considered as supplemental information.	1999/11791: Ebert, 1999 2011/1101715: Ebert, 2012 2012/1021122: Miles, 2012 (Kinetic evaluation of study Ebert)
BBA IV, 6-1, OECD Draft Test Guideline "Phototransformation of Chemicals in Water" Part A	*Aqueous photochemical degradation* Quantum yield Φ of direct phototransformation in water at Σ > 290 nm: 2.17. The calc. theoretical photolytic-half-lives of pyraclostrobin in the top layer of aqueous systems range from 17 days in April to 0.7 days in July. DT_{50} under lab. conditions: 0.06 d. 5 photodegradates >10% TAR: BF 500-11: 44.5% AR (21 d) BF 500-13: 16.8% AR (6 d) BF 500-14: 20.7% AR (3 h) BF 500-15: 26.6% AR (1 d) 500M58: 23.4% AR (1 d)	Reliable	1998/10257: Scharf, 1998 And 1999/11286: Scharf, 1999

[1] back-calculated as DT_{50} = DT_{90} / 3.32 according to FOCUS kinetics
[2] No reliable endpoints derived in kinetic evaluation.
[3] (t), (c) - tolyl or chlorophenyl-labeled test item used

[4] Persistence endpoint deviating from modelling endpoint given in brackets.
[5] DT_{90} not reported; calculated from SFO DT_{50} as $DT_{90} = DT_{50} \times 3.32$

11.1.1 Ready biodegradability

Author:	Reuschenbach P.
Title:	Determination of the biodegradability of BAS 500 F in the manometric respirometry test according to GLP, EN 45001 and ISO 9002
Date:	07.06.1999
Doc ID:	1999/10655
Guidelines:	EEC 92/69, OECD 301 F, ISO 9408
GLP:	yes
Validity:	Acceptable

11.1.1.1 Material and Methods

The aerobic biodegradability of pyraclostrobin was evaluated in the "Manometric Respirometry Test". Mixtures of the test substance at a concentration of 100 mg/L, a defined inorganic medium and a non-preadapted inoculum were incubated in a respirometer (Sapromat). The inoculum was activated sludge from laboratory wastewater treatment plants which were fed with municipal and synthetic sewage. The test vessels and appropriate controls were incubated and aerated at room temperature for up to 28 days. The oxygen used for the biodegradation of the test substance (biochemical oxygen demand, BOD) was continuously produced and measured by the test apparatus. For evaluation the measured BOD is compared to the calculated theoretical oxygen demand (ThOD).

11.1.1.2 Results and Discussion

After 28 days a degree of biodegradation of 0 – 10% (BOD of ThOD) was measured. The test substance was considered as poorly biodegradable and not readily biodegradable in this test.

11.1.1.3 Conclusion

Pyraclostrobin has to be considered as “not readily biodegradable”.

11.1.2 BOD_5/COD

No data available.

11.1.2.1 Hydrolysis

Author:	Scharf J.
Title:	Hydrolysis of BAS 500 F
Date:	20.01.1999
Doc ID:	1999/10060
Guidelines:	EEC 94/37, EPA 161-1
GLP:	yes
Validity:	acceptable
Previous evaluation:	In initial DAR (2001)

11.1.2.2 Material and Methods

Hydrolysis of pyraclostrobin was tested in aqueous buffer solutions at 50 °C at four different pH values (pH 4, 5, 7, and 9), and at 25 °C at three different pH values (pH 5, 7, 9). The concentration of pyraclostrobin in the buffer solutions was 0.5 mg/L and 1.0 mg/L for the tests at 25 °C and 50 °C, respectively. The solutions were incubated in the dark under sterile conditions. Sampling times for the test at 50 °C were 0, 1, 2, 3, 4, and 5 DAT, and for the test at 25 °C 0, 1, 3, 7, 15, 21, 24, and 30 days after treatment (DAT).

11.1.2.3 Results and Discussion

During hydrolysis at 25 °C and pH 5 and 7, the active substance was stable. At pH 9, a very slow decrease to 78 % TAR within 30 days could be observed. At all pH's, very small amounts of BF 500-5, BF 500-6 and BF 500-7 were produced sporadically. The results are summarized in Table 91.

At 50 °C and pH 9, the same degradation products were detected. Because of the higher temperature, their concentrations were accordingly higher. After 5 days, BF 500-5 amounted to 133 % TAR. BF 500-6 and BF 500-7 reached concentrations of 4.3 % TAR and 12.8 % TAR, respectively, within the same time. The results for the tolyl labelled active substance were comparable. In all tests, BF 500-3 was present as impurity already at 0 DAT.

No DT_{50}-values were calculated neither for acidic nor for alkaline conditions because they will exceed the period of reliable extrapolation (twice the duration of the studies).

Table 91: Recovery of radioactivity in % TAR and distribution of metabolites during hydrolysis of [14C]-chlorophenyl-labelled pyraclostrobin at 25 °C

pH	DAT	pyraclostrobin	BF 500-3 (500M07)	BF 500-5 (500M04)	BF 500-6 (500M01)	BF 500-7 (500M02)	others [1)]	sum
5	0	97.1	2.9					100.0
	1	90.5	4.7					95.2
	3	95.2	3.5		1.7			98.7
	7	92.5	4.5					97.0
	15	93.4	4.3			2.3	1.6	101.7
	21	84.5	3.7				5.0	93.2
	24	91.2	4.2				1.4	96.8
	30	88.7	3.6				1.4	95.4
7	0	90.7	6.4			1.3	1.6	100.0
	1	96.6	3.7				3.2	103.5
	3	95.3	6.9					102.2
	7	95.4	3.9					99.3
	15	94.6	4.3				1.0	99.8
	21	90.8	1.3	4.0			7.1	103.2
	24	91.1	4.9				4.7	100.7
	30	97.1	5.1				5.0	107.2
9	0	91.4	4.6	4.0				100.0
	1	83.0	5.4			2.9	6.2	97.6
	3	95.3	4.8					100.1

pH	DAT	pyraclostrobin	BF 500-3 (500M07)	BF 500-5 (500M04)	BF 500-6 (500M01)	BF 500-7 (500M02)	others [1)]	sum
	7	89.7	5.1			0.9		95.8
	15	92.0	3.1					95.1
	21	95.8	4.0					99.8
	24	91.8	4.7					96.5
	30	78.4	5.6		1.9	5.4		91.2

[1)] each < 4% TAR

11.1.2.4 Conclusions

The hydrolytic degradation of pyraclostrobin depends on the pH value. At pH 5 and 7, the active substance is stable. Under alkaline conditions (pH 9) a very slow degradation of the parent compound was observed at 25°C. Only at high temperatures (50 °C) and under alkaline conditions (pH 9) a comparatively faster degradation was observed, but this does not represent common environmental conditions.

11.1.3 Other convincing scientific evidence

No data available

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

No data available

11.1.3.2 Inherent and enhanced ready biodegradability tests

No data available

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

11.1.3.3.1 Aerobic mineralisation in surface water

Author:	Ebert D.,Possienke M.
Title:	^{14}C-BAS 500 F (Pyraclostrobin): Aerobic mineralisation in surface water
Date:	23.09.2013
Doc ID:	2013/1002741
Guidelines:	OECD 309 (April 2004)
GLP:	yes
Validity:	acceptable

The degradation of pyraclostrobin under aerobic aquatic conditions was investigated over a period of up to 63 days at 20 °C in the dark. Two test variants, pelagic test and suspended solid test, were investigated in parallel. Both test variants were performed with two different pyraclostrobin concentrations (10 µg L^{-1} and 50 µg L^{-1}) and two different ^{14}C-labelled test items (chlorophenyl-label and tolyl-label). The chlorophenyl-labelled test item contained the metabolite BF 500-3 as impurity.

The obtained results showed that pyraclostrobin was degraded very slowly in a pure water environment as provided in the pelagic test. More than 78 – 97 % TAR were still detectable as unchanged parent in the water

phase 63 days after treatment under dark conditions. The results were overall very comparable to the sterile test vessels. The degradation of pyraclostrobin under the pelagic test conditions was thus characterized by slow hydrolysis and formation of low amounts of cleavage products, of which BF 500-5 occurred at 5.6 - 10.9 % TAR. All other peaks never exceeded 2.1 % TAR.

In the suspended solid test, pyraclostrobin dissipated at a fast rate from the water phase (< 45 % TAR after 3 days) and adsorbed to the solid particles floating in the water. Despite the overall low amount of suspended solids, pyraclostrobin behaved as known from soil and sediment studies and converted quickly from extractable residues into non-extractable residues due to binding to the organic matrix.

In the water phase of the suspended solid test, the hydrolysis product BF 500-5 was detected in maximum amounts of 7.7 % TAR with the chlorophenyl-label. One peak in the tolyl-labelled treated test vessels (low concentration) reached 5.8 % TAR after 44 days (but only max. 2 % TAR in the high concentration test). It declined again to 2.1 % TAR after 63 days. Due to the low substance amounts, identification was not possible. All other peaks never exceeded 3.8 % TAR.

In the sediment extracts of the suspended solid test, besides BF 500-3 which was present in the treatment solution of the chlorophenyl-labelled test item, also the known soil and sediment metabolites BF 500-6 (≤ 2.3 % TAR) and BF 500-7 (≤ 2.6 % TAR) were formed. Other components did not exceed 0.5 % TAR at any sampling time.

The formation of volatiles in both test variants was generally low (< 5 % TAR at any time point), irrespective of test concentration or label position.

Overall, the degradation of pyraclostrobin was characterized by a low mineralization rate in both test variants irrespective of test concentration or label position. The amount of $^{14}CO_2$ never exceeded 5 % TAR within 63 days.

Kinetic analysis and calculations of DT_{50} and DT_{90} values for the pelagic and the suspended solid test were performed following the recommendations of the FOCUS Kinetics workgroup [FOCUS, 2006] to derive aquatic persistence and modeling endpoints. The analysis was done by a non-linear regression method (Iteratively Reweighted Least Squares) using the software package KinGUI version 2.

The $DegT_{50}$ values in the pelagic test ranged from 410 to 458 days for pyraclostrobin and from 11 to 29 days for BF 500-3. In the suspended solid test, the $DegT_{50}$ for pyraclostrobin for the whole system ranged from 26 to 28 days, while the $DisT_{50}$ for the water compartment was calculated to range from 7 to 10 days and for the suspended sediment from 44 to 47 days. The $DisT_{50}$ for BF 500-5 was 103 days when calculated with the high test concentration. For the low test concentration no adequate fit was achieved.

In general, pyraclostrobin hydrolyses only slowly under the pelagic test conditions, but adsorbs quickly to suspended solids, when available, and is then further degraded by formation of bound residues.

A summary of the DT_{50} and DT_{90} values of pyraclostrobin and its metabolite BF 500-3 for the pelagic test are given in Table 92. A summary of the DT_{50} and the DT_{90} values of pyraclostrobin for total system as well as for water and sediment separately determined for the suspended solid test is presented in Table 92. DT_{50} and DT_{90} values of the metabolite BF 500-5 for the suspended solid test are shown in Table 93 and Table 94.

Table 92: Summary of the kinetic evaluation of pyraclostrobin and its metabolite BF 500-3 for the pelagic test

Component	**Test system**		**Modeling endpoints**	
	Pelagic test	**Kinetic model**	**$DegT_{50}$ [d]**	**$DegT_{90}$ [d]**
Pyraclostrobin	High test concentration	SFO	410.4	1363
Pyraclostrobin	Low test concentration	SFO	458.1	1522
BF 500-3	High test concentration	SFO	28.6	94.8
BF 500-3	Low test concentration	SFO	10.7	35.6

Table 93: Summary of the kinetic evaluation of pyraclostrobin for the suspended solid test (Level P-I)

Test system	**Best-fit kinetics**			**Modeling endpoints**	
Total system	**Kinetic model**	**$DegT_{50}$ [d]**	**$DegT_{90}$ [d]**	**Kinetic model**	**$DegT_{50}$ [d]**
High test concentration	SFO	28.3	94.1	SFO	28.3
Low test concentration	SFO	26.4	87.7	SFO	26.4
Water compartment	**Kinetic model**	**$DisT_{50}$ [d]**	**$DisT_{90}$ [d]**	**Kinetic model**	**$DisT_{50}$ [d]**
High test concentration	DFOP	2.3	34.6	DFOP	10.4*
Low test concentration	FOMC	1.7	22.9	FOMC	6.9*
Suspended sediment compartment	**Kinetic model**	**$DisT_{50}$ [d]**	**$DisT_{90}$ [d]**	**Kinetic model**	**$DisT_{50}$ [d]**
High test concentration	SFO	46.8	155.5	SFO	46.8
Low test concentration	SFO	43.6	144.8	SFO	43.6
* Calculated according to FOCUS (DT_{50} = DT_{90}/3.32)					

Table 94: Summary of the kinetic evaluation of the metabolite BF 500-5 for the suspended solid test (Level M-I, chlorophenyl label)

Test system		**Modeling endpoints**		
Suspended solid test	**Kinetic model**	**$DisT_{50}$ [d]**	**$DisT_{90}$ [d]**	**Formation fraction [-]**
High test concentration	SFO	103.4	343.5	0.07
Low test concentration	-	No adequate fit could be achieved		

Conclusion

A pelagic test and a suspended solid test were performed under aerobic and dark conditions at nominal concentrations of 10 and 50 µg/L. In the pelagic test pyraclostrobin degraded very slowly with DT_{50} values between 410.4 d and 458.1 d (SFO). The major route of degradation under those conditions represented hydrolysis to cleavage products (BF 500-5 detected at max. 5.6 - 10.9 % TAR with the chlorophenyl-label, and trace amounts of several unknown peaks).

In the suspended solid test pyraclostrobin degraded faster with DT50 values between 26.4 d and 28.3 d (SFO) in the total system. Pyraclostrobin quickly adsorbed to the sediment.

However, it adsorbed and degraded very fast as soon as dissolved particles were available as shown in the suspended solid test. Even with low amounts of suspended sediment particles, it was quickly converted from extractable residues into non-extractable residues and thus tightly bound to the organic matrix.

The calculated $DegT_{50}$ values in the pelagic test were in the range from 410 to 458 days for pyraclostrobin and 11 to 29 days for BF 500-3. In the suspended solid test, for the whole system the $DegT_{50}$ ranged between 26 and 28 days, while the $DisT_{50}$ for the water compartment was calculated from 7 to 10 days and for the suspended sediment from 44 to 47 days. The $DisT_{50}$ for BF 500-5 was 103 days when calculated with the high test concentration. For the low test concentration no adequate fit was achieved.

11.1.3.3.2 Water sediment studies – dark systems

Author:	Staudenmaier H.
Title:	Degradation of BAS 500 F in aerobic aquatic environment
Date:	21.09.1999

Doc ID:	1999/11241
Guidelines:	SETAC Europe, BBA IV, 5-1, US-EPA, Subdivision N, 162-4
GLP:	yes
Validity:	acceptable

Material and Methods

The distribution and degradation of pyraclostrobin was studied in two natural systems of water and sediment. The water/sediment systems were taken from a pond (System A) and a pond-like side arm of a river (System B), respectively, both in Rhineland-Palatinate, Germany. Two radiolabelled forms of pyraclostrobin, chlorophenyl-[^{14}C] and tolyl-[^{14}C], were used and applied separately to the test systems.

Characteristics of the water/sediment systems are given in the table below.

Table 95: Characterisation of the water/sediment systems

Designation **Origin**		**System A** **Kastenbergheide** **Rhineland-Palatinate, FRG**	**System B** **Berghäuser Altrhein** **Rhineland-Palatinate, FRG**
Sediment	sand [%]	78	38
	silt [%]	4	42
	clay [%]	18	20
	textural class (German scheme)	clayey sand	silty clayey loam
	pH	7.1	7.3
	organic C [%]	0.8	8.3
	total N [%]	0.07	0.46
	total P [%]	0.01	0.11
	CEC [mVal/100g]	13.4	32.0
	ATP [µg/kg]	69.5	1568
	plate counts [cfu/g]		
	bacteria	4.5 x 10^7	2.6 x 10^8
	actinomycetes	4.0 x 10^5	5.3 x 10^6
	fungi	4.0 x 10^4	1.7 x 10^6
Water	pH	8.4	8.1
	hardness [mmol/l]	1.52	1.90
	TOC [mg/L]	10.0	6.4
	total N [mg/L]	1	1
	total P [mg/L]	2	<3

Glass test vessel were filled with about 200 g wet sediment (Kastenbergheide) or 120 g wet sediment (Berghäuser Altrhein). 290 mL water of the respective systems were added. This corresponded to a sediment layer of about 2.5 cm and a water layer of about 6 cm as required according to the BBA guideline IV, 5-1. After being filled with sediment and water, the systems were allowed to equilibrate for 28/29 days at incubation conditions (20 °C, dark, continuous aeration).

Pyraclostrobin was applied to the water at a rate of 30 µg a.s. per test vessel which corresponded to approx. 125 % of the maximum recommended rate of 250 g as/ha when related to a 30 cm deep water body.

For the isolation and identification of degradation products, some water/sediment systems were additionally treated at an application rate of 300 µg a.s. per test vessel. The test vessels were incubated in the dark at a temperature of 20 ± 2 °C for up to 100 days. Aeration was achieved by a stream of air over the water surface.

Samples were taken at 0 h, 6 h, and 1, 2, 7, 14, 30, 61, and 100 days after treatment.

Results and Discussion

The results from the two different radiolabels revealed no significant differences; therefore the differently radiolabelled replicates were averaged. The distribution and recovery of radioactivity from water/sediment

systems A and B is shown in the tables below.

Table 96: Material balance and distribution of radioactivity after application of [^{14}C]-pyraclostrobin to water/sediment system A (% TAR)

DAT	water	Sediment					CO_2	balance
		extractable residues			Bound residues	total		
		ACN/H_2O	ACN	total				
0	87.1	7.4	1.8	9.2	0.4	9.6	n.d.	96.7
0.25	74.4	15.5	4.2	19.7	1.0	20.7	0.0	95.1
1	60.2	27.1	7.2	34.2	3.4	37.6	0.0	97.9
2	50.0	21.6	12.8	34.4	11.2	45.6	0.0	95.7
7	24.6	32.4	20.2	52.6	16.8	69.5	0.0	94.1
14	15.6	44.3	15.4	59.7	22.4	82.2	0.0	97.8
30	6.9	32.3	15.9	48.2	37.2	85.5	0.5	92.8
61	3.5	19.6	16.8	36.4	53.1	89.4	4.6	97.5
100	2.1	15.5	14.0	29.5	61.8	91.3	4.1	97.5
103 s	4.1	57.8	21.4	79.2	15.0	94.2	n.d.	98.3

s = sterilised
n.d. = not determined

Table 97: Material balance and distribution of radioactivity after application of [14C]- pyraclostrobin to water/sediment system B (% TAR)

DAT	water	sediment					CO_2	balance
		extractable residues			bound residues	total		
		ACN/H_2O	ACN	total				
0	87.2	5.5	2.2	7.7	0.3	8.0	n.d.	95.3
0.25	63.3	22.2	9.3	31.4	1.2	32.7	0.0	95.9
1	38.5	40.4	16.5	56.9	2.7	59.6	0.0	98.1
2	24.8	45.0	19.7	65.1	4.2	69.4	0.0	94.1
7	8.9	55.7	21.4	77.1	8.0	85.2	0.0	94.1
14	3.7	52.6	27.9	80.5	14.2	94.7	0.0	98.3
30	2.7	38.7	33.3	72.0	20.3	92.3	0.3	95.3
61	3.2	28.6	29.2	57.9	33.7	91.6	0.8	95.6
100	2.8	20.7	17.4	38.1	54.1	92.2	4.6	99.6
103 s	2.8	58.2	31.7	90.0	4.9	94.9	n.d.	97.6

s = sterilised
n.d. = not determined

The radioactivity moved quite fast from the water to the sediment. The radioactivity in the water decreased to less than 25 % TAR within 7 days in system A and within 2 days in system B. A further decrease to less than 3 % TAR after 100 days was observed in both systems. In the sediment a corresponding increase was seen which accounted for more than 90 % TAR at the end of the incubation period. Mineralisation was low in both systems with 4.6 % TAR and no other volatile degradates were detected.

High amounts of bound residues were formed in the sediment which accounted for up to 61.8 % TAR in system

A and 54.1 % TAR in system B. These residues were fractionated into humins, humic acids and fulvic acids. No detectable amounts of pyraclostrobin were released from the bound residues.

Although the degradation seemed to proceed mainly in the sediment in both systems, some differences were observed between the two water/sediment systems: In system A significant degradation of the test substance was detectable from 2 DAT on, which proceeded continuously down to 6.5 % TAR at 100 DAT. Metabolite BF 500-3 was formed in moderate amounts up to 11.6 % TAR and BF 500-6 and BF 500-7 were formed up to approx. 6.5 % TAR each. In contrast, in system B a pronounced decrease of the active substance was detected from 7 DAT on which was accompanied by a corresponding increase of BF 500-3 up to 67.7 % TAR. This metabolite was degraded again and amounted to 28.5 % TAR after 100 days. BF 500-6 and BF 500-7 were not detected in system B. Other metabolites were only detected in trace amounts in both systems.

In the water of both water/sediment systems, the active substance was found to be the only radiolabelled compound except trace amounts of BF 500-3. All other metabolites were detected only in the sediment. They were formed soon after significant amounts of the active substance had moved into the sediment.

Degradation of the test substance in the sterilised test vessels was much slower than in the viable samples. After 103 days, 83.3 % TAR (system A) and 92.8 % TAR (system B) was still unchanged test substance. Metabolites were not detected at all and final degradation to CO_2 and bound residues was much reduced indicating the involvement of microbial processes in the degradation of pyraclostrobin.

For a kinetic evaluation of the experimental data see the following study *Wiedemann, 2013*.

Conclusion

The fate of pyraclostrobin was investigated for up to 100 d in two water/sediment systems Kastenbergheide and Berghäuser Altrhein. Mineralisation amounted to maxima of 4.6 % AR. Bound residues amounted to 61.8 % and 54.1 % AR. Maximum concentrations of pyraclostrobin in the sediment were 52.5 % at day 14 and 62.1 % at day 2, respectively.

Three metabolites were identified in the study:

- the major metabolite BF 500-3 accounted for a maximum of 67.6 % AR at day 14 (1.9 % in water, 65.7 % in sediment, Berghäuser Altrhein).
- the major metabolite BF 500-6 accounted for a maximum of 6.5 % AR at the last sampling drawings at days 61 and 100 (only in sediment, Kastenbergheide).
- the metabolite BF 500-7 accounted for a maximum of 6.3 % AR at day 61 (only in sediment, Kastenbergheide, only once > 5 %).

A new kinetic evaluation of this study has been submitted (*Wiedemann, 2013*) for the renewal of the EU approval of pyraclostrobin.

11.1.3.3.3 Water sediment studies – kinetic evaluation

Author:	Wiedemann G.
Title:	Kinetic evaluation of BAS 500 F - Pyraclostrobin in water/sediment systems under aerobic conditions
Date:	03.05.2013
Doc ID:	2012/1165029
Guidelines:	FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Sanco/10058/2005 version 2.0 434 pp.

GLP:	no
Validity:	acceptable

The aim of the study was to evaluate the dissipation and degradation kinetics of pyraclostrobin in two aerobic water/sediment systems with two different labels [*Staudenmaier H., 1999; BASF DocID 1999/11241*] and to derive persistence and modeling endpoints according to the recommendations of FOCUS kinetics.

In the laboratory study the degradation of pyraclostrobin was investigated over a period of 100 days in two water/sediment systems called System A and B (a pond and a pond-like side arm of a river located in Southern Germany). Two radio-labels of the active substance were used in the study and were considered independently as replicates in the kinetic evaluation. The experimental data were evaluated using single first order (SFO), first-order multi-compartment (FOMC), double first-order in parallel (DFOP) and hockey stick (HS) kinetic models at the evaluation levels P-I (one-compartment approach) and P-II (two-compartment approach).

The experimental data of the parent substance used as model input values for the kinetic evaluations are given in Table 98 and Table 99.

Table 98: Model input for pyraclostrobin – System A

Time [d]	**Concentration [% Total Applied Radioactivity]**					
	Chlorophenyl-label			**Tolyl-label**		
	Water	**Sediment**	**Whole system**	**Water**	**Sediment**	**Whole system**
0	95.1[a]	0.0[b]	95.1	97.6[a]	0.0[b]	97.6
0.25	72.9	19.5	92.5	75.6	18.5	94.0
1	61.9	31.5	93.4	58.5	34.1	92.6
2	49.6	34.4	84.0	50.5	29.8	80.3
7	22.5	34.9	57.4	26.7	44.2	70.9
14	16.0	51.3	67.3	14.9	53.7	68.7
30	6.7	33.7	40.4	7.1	31.4	38.4
61	not reported[c]	13.3	13.3	3.5	20.8	24.3
100	not analyzed	6.5	6.5	not analyzed	6.4	6.4

[a] sum of radioactivity found in all compartments (sum of parent and metabolites)
[b] set to zero as recommended by FOCUS for the P II-level
[c] poor signal to noise ratio
Sediment values before day 14 (max. occurrence in sediment) were not used, when fitting only degradation in sediment at the P I-level.

Table 99: Model input for pyraclostrobin – System B

Time [d]	**Concentration [% Total Applied Radioactivity]**					
	Chlorophenyl-label			**Tolyl-label**		
	Water	**Sediment**	**Whole system**	**Water**	**Sediment**	**Whole system**
0	95.0[a]	0.0[b]	95.0	95.0[a]	0.0[b]	95.0
0.25	67.2	26.7	93.9	59.0	33.8	92.8
1	39.6	53.7	93.2	37.4	57.0	94.4
2	25.9	62.1	88.0	23.5	62.0	85.5
7	8.1	25.4	33.4	9.5	70.0	79.5
14	1.8	15.0	16.8	1.6	14.5	16.2

30	1.0	8.1	9.1	0.6	9.7	10.3
61	not reported[c]	9.4	9.4	1.1	7.1	8.2
100	not analyzed	7.4	7.4	not analyzed	11.8	11.8

[a] sum of radioactivity found in all compartments (sum of parent and metabolites)
[b] set to zero as recommended by FOCUS for the P II-level
[c] poor signal to noise ratio
Sediment values before the maximum occurrence were not used, when fitting only degradation in sediment at the P I-level.

Results and Discussion

For modeling endpoints, the initial fit was performed using SFO kinetics. If the fit was not satisfactory, FOMC, DFOP and HS kinetics were tested. For persistence endpoints, SFO and FOMC kinetics were tested in a first step; if SFO was not acceptable or worse than FOMC, DFOP and HS kinetics were tried in addition. Graphical presentations of the tested kinetic models and the results of the χ^2 - test and all other statistical endpoints used in the decision-making process are given in the original study report.

Level P-I

The evaluation of persistence and modeling endpoints of both test systems for both labels at P-I level showed that dissipation in the water phase could be well described by bi-phasic kinetics (FOMC: System A, chlorophenyl label; DFOP: System A, tolyl label, and System B, both labels), while dissipation from sediment and degradation in the whole system were best described by SFO kinetics (System A, both labels). For system B, none of the models was acceptable for dissipation from sediment and degradation in the whole system.

An overview of the estimated persistence and modeling endpoints for pyraclostrobin from both water/sediment systems is given in Table 100 and Table 101.

Table 100: Persistence endpoints at P-I level

System, Label	Water		Sediment		Whole system	
	DT_{50}/DT_{90} [d]	Kinetic model	DT_{50}/DT_{90} [d]	Kinetic model	DT_{50}/DT_{90} [d]	Kinetic model
System A, chlorophenyl-label	2.13 / 28.8	FOMC	25.7 / 85.5	SFO	23.3 / 77.4	SFO
System A, tolyl-label	2.30 / 17.5	DFOP	29.8 / 98.9	SFO	26.8 / 89.1	SFO
System B, chlorophenyl-label	0.636 / 6.19	DFOP	-*	-	-*	-
System B, tolyl-label	0.443 / 6.28	DFOP	-*	-	-*	-

* The data could not reliably be described by the models and should therefore not be considered for the assessment. Note, that in the study report a default of 1000 days was reported, which, however, is not in line with FOCUS and should therefore be corrected.

Table 101: Modeling endpoints at P-I level

System, Label	Water		Sediment		Whole system	
	DT_{50} [d]	Kinetic model	DT_{50} [d]	Kinetic model	$DegT_{50}$ [d]	Kinetic model

System A, chlorophenyl-label	8.67*	FOMC	25.7	SFO	23.3	SFO
System A, tolyl-label	5.27*	DFOP	29.8	SFO	26.8	SFO
System B, chlorophenyl-label	1.86*	DFOP	-**	-	-**	-
System B, tolyl-label	1.89*	DFOP	-**	-	-**	-

* Back-calculated from DT_{90} ($DT_{50} = DT_{90} / 3.32$) as required by FOCUS kinetics.
** The data could not reliably be described by the models and should therefore not be considered for the assessment. Note, that in the study report a default of 1000 days was reported, which, however, is not in line with FOCUS and should therefore be corrected.

Level P-II

Degradation of pyraclostrobin in water and sediment as well as partitioning between both phases was analyzed according to the P-II level kinetic concept (two-compartment approach) of the FOCUS guidance document. A compartment model was used and SFO kinetics were considered for the transfer and degradation rates.

P-II level analysis was performed for both systems and both labels, however, the results showed a poor fit to the measured data. For system A the fits were visually good for water and sediment, but statistically poor. While for system B (chlorophenyl label) the fits were visually good and statistically poor for water, it was the contrary for sediment. The fit in water and sediment did not reflect sufficiently the substance behavior in the system, probably because the degradation in sediment seemed to stop soon after the beginning of the study. Repeating the optimization with different starting values did not change the estimated values. The visual fit for water and sediment was moderate for system B (tolyl label), but statistically poor.

The F_{sed} test was performed for both systems and labels, but failed in all cases. No reliable fits for the level P-II could be obtained.

The experimental data on pyraclostrobin in both test systems (Systems A and B) and for both labels (chlorophenyl- and tolyl-labels) were evaluated at Level P-I and Level P-II. For system B no reliable endpoints could be derived for the sediment compartment and for the whole system, for both labels. As a consequence a "default" value was assumed, which, however, is not correct following the FOCUS kinetics guidance. According to FOCUS kinetics a case-by-case decision should be made if reliable endpoints cannot be derived with the recommended standard procedures. Moreover, for System B a $DegT_{90}$ in the whole system of approximately 30 days can be estimated visually, which is considerably shorter than the endpoint calculated for System A. Hence, the results of the kinetic evaluation of System A represent the worst-case of the two w/s systems investigated. Consequently, only the results of the kinetic evaluation of System A are to be considered as worst-case for further usage.

Reliable $DegT_{50}$ values for the whole system were derived for System A for both labels which range from 23.3 to 26.8 days. This gives a geometric mean value of 25.0 days. When pooling both labels for the kinetic evaluation, the same value of 25.0 days is retrieved (see below). The analysis at the P-II level did not result in reliable fits.

Evaluation of the $DegT_{50}$ of the total system for system A when pooling both labels – SFO kinetics

The model CAKE v3.3 was used to kinetically analyze the total system $DegT_{50}$ using SFO kinetics. The raw data including the visual fits and statistical indices are given below. The visual fit is good, and the kinetic parameters are statistically acceptable (Chi-square 6.5%, p-value of the degradation rate $k < 0.05$). The $DegT_{50}$ of 25.0 days is the same value as the geometric mean of the individual labels.

Conclusion

Reliable endpoints for the sediment and the total system could only be derived for system A. Pyraclostrobin degraded with DT_{50} values of 23.3 d and 26.8 d and DT_{90} values of 77.4 d and 89.1 d following SFO kinetics

for the two labels in system A. This gives a geometric mean value of 25.0 days for the DT_{50}. When pooling both labels for the kinetic evaluation, the same value of 25.0 days is retrieved for the DT_{50}. The dissipation from the water phase followed biphasic kinetics in both systems, with $DissT_{50}$ < 3 days and $DissT_{90}$ < 30 days. The dissipation from the sediment followed SFO in system A, with $DissT_{50}$ < 30 days and $DissT_{90}$ < 100 days.

11.1.3.3.4 Water sediment studies – irradiated conditions

Author:	Ebert D.
Title:	Degradation of BAS 500 F in aerobic aquatic environment under irradiated conditions
Date:	03.12.1999
Doc ID:	1999/11791
Guidelines:	US-EPA, Subdivision N, 162-4
GLP:	yes
Validity:	not acceptable, deviating from current guideline OECD 308

Material and Methods

This study was initiated after it became obvious that the degradation of pyraclostrobin in water is strongly dependent on light conditions. The aqueous photolysis study showed that pyraclostrobin is quickly degraded under irradiated conditions forming numerous rearrangement and breakdown products. In the water/sediment study, however, it could be shown that pyraclostrobin quickly binds to the sediment. Since in natural water/sediment systems (rivers, lakes etc.) both factors, photolysis and sediment adsorption, will influence the degradation of pyraclostrobin simultaneously, this additional study was designed where both factors were combined.

The water/sediment system taken for this study was of the same origin as one of the systems used for the aerobic aquatic metabolism (Kellmetschweiher, also named Kastenbergheide). The water/sediment characteristics are summarised in Table 102.

Table 102: Characterisation of the water/sediment Kellmetschweiher

water/sediment designation **origin**		**Kellmetschweiher** **Schifferstadt, Rhineland Palatinate, Germany**
water	**pH at site of sampling**	**8.6**
sediment	textural class (German scheme)	sand / clayey sand
	clay [%]	5
	silt [%]	2
	sand [%]	93
	organic C [%]	0.4
	pH ($CaCl_2$)	7.5

Test vessels were filled with about 1.5 cm sediment and a water layer of about 15 cm height. Both radiolabels of pyraclostrobin, chlorophenyl-[^{14}C] and tolyl-[^{14}C], were applied separately to the test vessels. Pyraclostrobin was applied at a rate of 244 µg per test vessel for the chlorophenyl-label and 217 µg for the tolyl-label. This roughly corresponds to twofold the maximum recommended application rate of 250 g active substance/ha, when assuming direct overspray of a 30 cm deep water body.

The treated water/sediment systems were incubated in a climatic chamber (phytotron), where light (simulated sun light) and temperature conditions of Central Europe were simulated (daily exposure and temperature cycles in the period of May 17th – July 18th). This period represents the main application period for pyraclostrobin within the year. Water and sediment samples were taken up to 62 days after treatment.

Results and Discussion

The distribution of radioactivity and material balance in the water/sediment system is shown in Table 103 for both labels.

Table 103: Distribution of radioactivity and material balance in the water/sediment system after application of ^{14}C-pyraclostrobin and incubation under realistic light and temperature conditions

time after treatment	% TAR				
	water	sediment extractable residues	sediment bound residues	Sediment total	material balance (water + sediment)
chlorophenyl-label					
0 h	88.0	n.s.	n.s.	n.s.	n.s.
3 h	89.4	n.s.	n.s.	n.s.	n.s.
6 h	87.1	n.s.	n.s.	n.s.	n.s.
9 h	83.7	n.s.	n.s.	n.s.	n.s.
1 d	82.8	10.6	0.5	11.1	93.9
2 d	76.7	n.s.	n.s.	n.s.	n.s.
3 d	72.0	18.6	3.5	22.1	94.1
7 d	61.1	24.6	8.5	33.1	94.2
10 d	55.8	n.s.	n.s.	n.s.	n.s.
14 d	50.2	24.6	21.1	45.7	95.9
21 d	45.1	n.s.	n.s.	n.s.	n.s.
30 d	42.0	22.5	23.1	45.7	87.7
45 d	37.5	19.7	25.9	45.6	83.1
62 d	31.4	18.1	27.6	45.7	77.1
tolyl label					
0 h	89.4	n.s.	n.s.	n.s.	n.s.
3 h	91.4	n.s.	n.s.	n.s.	n.s.
6 h	90.6	n.s.	n.s.	n.s.	n.s.
9 h	83.8	n.s.	n.s.	n.s.	n.s.
1 d	81.2	9.9	0.4	10.3	91.4
2 d	80.9	n.s.	n.s.	n.s.	n.s.
3 d	78.4	18.1	1.8	19.9	98.3
7 d	69.1	25.6	4.5	30.1	99.1
10 d	63.4	n.s.	n.s.	n.s.	n.s.
14 d	59.6	24.7	13.6	38.2	97.8
21 d	57.3	n.s.	n.s.	n.s.	n.s.
30 d	55.9	26.6	14.3	40.9	96.9
45 d	51.5	24.2	19.7	43.9	95.3
62 d	46.2	21.5	25.5	47.0	93.2

n.s. = not sampled

The results of this study clearly show that pyraclostrobin follows two major dissipation and degradation pathways in a natural water system. When reaching water, pyraclostrobin undergoes a very fast photolytical transformation forming many breakdown products and polar degradates (Table 104 and Table 105), and simultaneously, it adsorbes very fast to the sediment where it is finally bound to the sediment matrix.

HPTLC analysis revealed that three major metabolites (> 10 % TAR) were formed in the water phase (BF 500-11, BF 500-13, BF 500-14). All three metabolites are already known from the aqueous photolysis study. Two metabolites which occured during the aqueous photolysis study also in amounts > 10 % (BF 500-15, 500M58) could not be detected at any sampling time.

In the sediment, pyraclostrobin is quickly de-methoxylated forming the metabolite BF 500-3 which reached a maximum of 17 % TAR. Because of the low water solubility and high K_{oc}-value BF 500-3 is not supposed to move from the sediment into the water. It is degraded further in the sediment and finally, the radioactivity is bound to the sediment matrix. The water metabolites are found in the sediment only in very low amounts.

In contrast to the tolyl-label, the chlorophenyl-label shows some very polar unidentified components in the water phase, reaching up to 10-1 3 % TAR. However, when analysing the samples by HPLC with a special column for polar substances, this polar region is separated into several components each below 10 % TAR. Since these polar components could not be detected with the tolyl-label, it can be concluded that they are various breakdown products derived from the chlorophenyl moiety which was split off from pyraclostrobin. With the chlorophenyl-label, the sum of radioactivity in water and sediment declined to 77 % TAR after 62 days which indicates a mineralization of about 23 % TAR. This finding is in agreement with the results of the aqueous photolysis study, where also a significant higher mineralization rate was observed with the chlorophenyl-label than with the tolyl-label.

Table 104: HPLC analysis of the water samples and sediment extracts after application of ^{14}C-pyraclostrobin to a water/sediment system and incubation under realistic light and temperature conditions (chlorophenyl-label)

time after treatment	**% TAR**							
	total	**Unknown polars Rf 0.00**	**Unknown polars Rf 0.01**	**BF 500-14 (500M76) Rf 0.18**	**unknown Rf 0.27**	**Pyraclostrobin Rf 0.80**	**BF 500-3 (500M07) Rf 0.89**	**others***
water								
0 h	88.0	0.2	0.1	0.3		82.7	2.2	2.5
3 h	89.4	0.8	0.1	1.5		80.7	2.6	3.7
6 h	87.1	0.5		1.5		79.3	2.6	3.2
9 h	83.7	0.6		2.2		75.0	2.6	3.3
1 d	82.8	1.6		3.9	0.3	69.1	2.9	5.0
2 d	76.7	1.6		5.9	0.2	58.2	3.0	7.7
3 d	72.0	3.0		9.0	0.5	46.2	2.9	10.4
7 d	61.1	5.1	4.6	10.4	0.8	28.3	2.5	9.3
10 d	55.8	7.8	9.9	11.1	1.2	14.9	2.1	8.9
14 d	50.2	6.6	7.2	11.4	1.7	12.5	1.9	9.0
21 d	45.1	9.3	9.2	8.1	3.3	3.8	3.0	8.3
30 d	42.0	10.2	12.5	4.2	4.5	0.7	3.3	6.6
45 d	37.5	7.3	10.5	2.8	5.8		3.1	8.0

time after treatment	% TAR							
	total	Unknown polars Rf 0.00	Unknown polars Rf 0.01	BF 500-14 (500M76) Rf 0.18	unknown Rf 0.27	Pyraclostrobin Rf 0.80	BF 500-3 (500M07) Rf 0.89	others*
sediment								
1 d	10.6			0.1		9.5	0.7	0.3
3 d	18.6	0.1		0.4	0.1	15.6	1.6	0.9
7 d	24.6	0.1		0.7	0.4	17.5	4.1	1.7
14 d	24.6	0.5		0.7	0.9	9.7	10.0	2.8
30 d	22.5	0.6		0.5	1.5	0.8	15.9	3.1
45 d	19.7	0.6	0.2	0.7	1.7	0.4	13.2	3.0
62 d	18.1	0.5	0.1	0.5	1.6	0.3	12.2	2.9

* sum of up to 13 peaks, each of them ≤ 3% TAR

Table 105: HPLC analysis of the water samples and sediment extracts after application of ^{14}C-pyraclostrobin to a water/sediment system and incubation under realistic light and temperature conditions (tolyl-label)

time after treatment	% TAR								
	total	BF500-14 (500M76) Rf 0.18	Unknown Rf 0.26	BF 500-11 (500M60) Rf 0.31	BF 500-13 (500M62) Rf 0.44	BF 500-12 (500M59) Rf 0.27	Pyraclostrobin Rf 0.80	BF 500-3 (500M07) Rf 0.89	others*
water									
0 h	89.4	0.1	0.1	0.2			85.2	2.4	1.5
3 h	91.4	1.3	0.1	0.9		0.8	84.0	2.7	1.6
6 h	90.6	1.4	0.1	1.1		0.9	82.4	2.8	1.9
9 h	83.8	1.7	0.2	1.2		1.2	75.5	2.6	1.5
1 d	81.2	2.7	0.2	2.1	0.4	1.7	68.7	2.6	2.8
2 d	80.9	4.7	0.3	3.7	0.6	3.1	61.0	3.0	4.4
3 d	78.4	6.6	0.5	5.7	1.2	3.9	51.0	3.0	6.5
7 d	69.1	8.5	0.7	7.8	2.2	3.3	34.2	2.7	9.7
10 d	63.4	10.8	1.3	10.4	3.7	2.2	17.3	2.3	15.4
14 d	59.6	9.7	1.6	10.3	4.1	1.8	14.0	2.4	15.1
21 d	57.3	8.6	3.0	11.4	7.0	1.0	5.4	3.3	17.6
30 d	55.9	5.6	4.7	10.5	10.5		2.1	5.0	17.7
45 d	51.5	2.3	5.9	5.5	14.0	0.6	0.8	4.7	17.6
62 d	46.2	1.7	5.9	3.9	15.7	0.9	0.9	4.1	13.1
Sediment									
1 d	9.9	0.1				0.1	8.9	0.6	0.2
3 d	18.1	0.3	0.1		0.1	0.2	15.0	1.4	0.9
7 d	25.6	0.5	0.3	0.1	0.4	0.3	18.3	4.0	1.7
14 d	24.7	0.6	0.8	0.2	0.8	0.2	6.4	12.4	3.2
30 d	26.6	0.4	1.7	0.3	1.8	0.1	0.9	16.9	4.7

time after treatment	% TAR								
	total	BF500-14 (500M76) Rf 0.18	Unknown Rf 0.26	BF 500-11 (500M60) Rf 0.31	BF 500-13 (500M62) Rf 0.44	BF 500-12 (500M59) Rf 0.27	Pyraclostrobin Rf 0.80	BF 500-3 (500M07) Rf 0.89	others*
45 d	24.2	0.6	1.9	0.5	2.1	0.2	0.5	14.3	4.3
62 d	21.5	0.5	1.8	0.6	1.9	0.3	0.3	12.7	3.8

* sum of up to 15 peaks, each of them <5% TAR

For a kinetic evaluation of the experimental data see the following study *Miles, 2013*.

Conclusion

The water/sediment study conducted under light conditions is helpful to understand the influence of light on the degradation and distribution of pyraclostrobin in aquatic systems. The entire evaluation of the active substance will be however done on the base of the study conducted in the dark, according to the current guideline.

Four major metabolites were identified in the study, which require a relevance assessment:

- BF 500-11 accounted for a maximum of 11.4% AR at day 21 in the water phase.
- BF 500-13 accounted for a maximum of 15.7% AR at day 62 in the water phase.
- BF 500-14 accounted for a maximum of 11.4% AR at day 14 in the water phase.
- BF 500-3 accounted for a maximum of 16.9% AR at day 30 in the sediment phase.

11.1.3.3.5 Water sediment studies – kinetic evaluation of irradiated study

Author:	Miles B.
Title:	Kinetic evaluation of BAS 500 F in water/sediment systems under aerobic conditions
Date:	03.02.2012
Doc ID:	2012/1021122
Guidelines:	FOCUS Kinetics Report SANCO/10058/2005 ver. 2.0
GLP:	no
Validity:	acceptable (but evaluated study *Ebert, 1999* not acceptable)

The aim of the study was to evaluate the dissipation and degradation kinetics of pyraclostrobin in aerobic water/sediment systems under irradiated conditions [*Ebert, 1999; BASF DocID 1999/11791*] and to derive modeling endpoints to be used in FOCUS surface water modeling . The fate and behavior of pyraclostrobin was investigated over a period of up to 62 days in one natural water/sediment system (Kellmetschweiher pond, Germany). Two radio-labels of the active substance were used in the study and were treated as replicates for the kinetic evaluation.

Kinetic evaluations were performed for pyraclostrobin and four of its metabolites (BF 500-3, BF 500-11, BF 500-13 and BF 500-14) considering the different levels proposed by the FOCUS Kinetics Guidance [*FOCUS (2006)*]. For the parent substance, the analysis at P-I level (one compartment approach) was done for degradation in the whole system as well as the respective dissipations from the water and sediment phases of the test system. At the P-II level (two-compartment approach), the kinetic analysis considered the degradation in water and sediment taking into account the partitioning between the two phases.

For the metabolites, dissipation calculations were performed at level M-I, although the considered radio-labels and system compartments differed for each metabolite.

As the purpose of the study was to derive modeling endpoints, only the single first order (SFO) kinetic model was considered as long as acceptable results were obtained in line with the FOCUS Kinetics Guidance [*FOCUS (2006)*]. The metabolite kinetics were described with the SFO kinetic model.

The dissipation kinetics were evaluated for the metabolites BF 500-11 and BF 500-14 in the water compartment, for BF 500-3 in the sediment compartment, and for all three metabolites in the total system.

Degradation kinetics for the water/sediment system were evaluated for the metabolites BF 500-3, BF 500-11, and BF 500-14. The metabolite BF 500-13 could not be evaluated in either compartment.

The appropriateness of a distinct kinetic model to describe degradation can be tested with the following checks recommended by *FOCUS (2006)*:

Visual assessment of goodness-of-fit

Estimation of the error percentage at which the χ^2 test is passed [*Equation 6-2 in FOCUS (2006)*]

t-test to evaluate whether estimated degradation parameters differ from zero.

A kinetic model is considered appropriate if the residuals are randomly distributed, the χ^2 - error value is < 15 % and the estimated degradation parameters differ from zero as outlined by *FOCUS (2006)*.

Where possible, replicate measurements (chlorophenyl- and tolyl-label) for each time point were used in the parameter estimation for the parent compound.

The experimental data of the parent substance used as model input values for the kinetic evaluations are given in Table 106.

Table 106: Model input for pyraclostrobin and metabolites

Time [d]	Concentration [% Total Applied Radioactivity]										
	Pyraclostrobin			BF 500-3		BF 500-11		BF 500-13		BF 500-14	
	Water	Sed.	Sys.	Sed.	Sys.	Water	Sys.	Water	Sys.	Water	Sys.
0 (c)	88.0*				2.2**					0.3	0.3**
0 (t)	89.4*				2.4**	0.2	0.2**		0.0	0.1	0.1**
0.125 (c)	80.7									1.5	
0.125 (t)	84.0					0.9				1.3	
0.25 (c)	79.3									1.5	
0.25 (t)	82.4					1.1				1.4	
0.375 (c)	75.0									2.2	
0.375 (t)	75.5					1.2				1.7	
1 (c)	69.1	9.5	78.6	0.7	3.6					3.9	4.0
1 (t)	68.7	8.9	77.6	0.6	3.2	2.1	2.1	0.4	0.4	2.7	2.8
2 (c)	58.2									5.9	
2 (t)	61.0					3.7		0.6		4.7	
3 (c)	46.2	15.6	61.8	1.6	4.5					9.0	9.4
3 (t)	51.0	15.0	66.0	1.4	4.4	5.7	5.7	1.2	1.3	6.6	6.9
7 (c)	28.3	17.5	45.8	4.1	6.6					10.4	11.1
7 (t)	34.2	18.3	52.5	4.0	6.7	7.8	7.9	2.2	2.6	8.5	9.0
10 (c)	14.9									11.1	
10 (t)	17.3					10.4		3.7		10.8	
14 (c)	12.5	9.7	22.2	10.0	11.9					11.4	12.1
14 (t)	14.0	6.4	20.4	12.4	14.8	10.3	10.5	4.1	4.9	9.7	10.3

Time [d]	Concentration [% Total Applied Radioactivity]										
	Pyraclostrobin			BF 500-3		BF 500-11		BF 500-13		BF 500-14	
	Water	Sed.	Sys.	Sed.	Sys.	Water	Sys.	Water	Sys.	Water	Sys.
21 (c)	3.8									8.1	
21 (t)	5.4					11.4		7.0		8.6	
30 (c)	0.7	0.8	1.5	15.9	19.2					4.2	4.7
30 (t)	2.1	0.9	3.0	16.9	21.9	10.5	10.8	10.5	12.3	5.6	6.0
45 (c)		0.4	0.4	13.2	16.3					2.8	3.5
45 (t)	0.8	0.5	1.3	14.3	19.0	5.5	6.0	14.0	16.1	2.3	2.9
62 (c)		0.3	0.3	12.2	15.7					1.6	2.1
62 (t)	0.9	0.3	1.2	12.7	16.8	3.9	4.5	15.7	17.6	1.7	2.2

Results and Discussion

Level P-I

The dissipation in the water and sediment compartments and the total system degradation could be well described by the SFO kinetic model (Table 107) without apparent significant systematic deviations in the residual errors. The fitted parameter values with their associated statistical attributes are given in Table 108. An overview of the estimated modeling endpoints for pyraclostrobin at Level P-I is given in Table 109.

Table 107: Evaluation of SFO kinetic models for pyraclostrobin at P-I level

	Number of measurements used for fitting	χ^2 error	Visual fit
BAS 500 F Whole system	16	4.568	Excellent
BAS 500 F Water	24	5.346	Excellent
BAS 500 F Sediment	8	3.300	Excellent

Table 108: Fitted parameter values and statistical assessment for pyraclostrobin at P-I level

	Parameter	Estimated value	Std error	type I error rate
BAS 500 F Whole system	M(0)	87.65	1.42	<0.001
	k	9.60E-02	4.27E-03	<0.001
BAS 500 F Water	M(0)	83.18	0.94	<0.001
	k	0.155	6.89E-03	<0.001
BAS 500 F Sediment	M(0)	17.93	0.63	<0.001
	k	0.117	1.07E-02	<0.001

Table 109: Modeling endpoints for pyraclostrobin at P-I level

	DT_{50} [days]	$DegT_{50}$ [days]
BAS 500 F Whole system	-	7.22
BAS 500 F Water	4.47	-
BAS 500 F Sediment	5.93	-

Level P-II

Degradation of pyraclostrobin in water and sediment as well as partitioning between both phases was analyzed according to the P-II level kinetic concept (two-compartment approach) of the FOCUS guidance document

[*FOCUS (2006)*]. A compartment model was used and SFO kinetics were considered for the transfer and degradation rates.

Good visual fits were obtained with SFO kinetics for both the water and sediment compartments. The standard errors were low for all of the estimated parameters and the t-test was passed in all cases (Table 110).

Table 110: Evaluation of SFO kinetic model for pyraclostrobin at P-II level with fitted parameter values

	χ^2 error	**Visual fit**	**Parameter**	**Estimated value**	**Std error**	**type l error rate**
BAS 500 F – Water	3.0	Good	M(0)	87.71	1.13	<0.001
			k_{water}	9.24E-02	1.64E-02	<0.001
			$r_{W\rightarrow S}$	0.143	1.45E-02	<0.001
BAS 500 F – Sediment	12.0	Good	M(0)	Fixed to 0		
			k_{sed}	1.07E-01	4.22E-02	0.011
			$r_{S\rightarrow W}$	0.227	4.68E-02	<0.001

The results obtained for the SFO kinetic model at Level P-II can be considered acceptable and the estimated degradation rates can be used as modeling endpoints. The $DegT_{50}$ values are given in Table 111.

Table 111: Modeling endpoints for pyraclostrobin at P-II level

	$DegT_{50}$ [days]
Water	7.50
Sediment	6.48

Level M-I

Five metabolites were found in the water/sediment systems: BF 500-3, BF 500-11, BF 500-12, BF 500-13 and BF 500-14. Metabolite BF 500-12, however, was not evaluated because the total occurrence in water and sediment was below 5% TAR. Both dissipation and degradation of the metabolites were studied at the M-I level according to the FOCUS guidance. The metabolite BF 500-13 could not be evaluated on the basis of the experimental data.

Dissipation

The dissipation kinetics were evaluated for the metabolites BF 500-11 and BF 500-14 in the water compartment and for BF 500-3 in the sediment compartment, and for all three metabolites in the whole system. The dissipation in the water and sediment compartments for the metabolites could be adequately described by the SFO kinetic model without apparent significant systematic deviations in the residual errors and with the t-test passed in all cases (Table 112; Table 113). The resulting modeling endpoints are given in Table 114.

Table 112: Evaluation of SFO kinetic models for metabolite dissipation at M-I level

	Number of measurements used for fitting	**χ^2 error**	**Visual fit**
BF 500-3 Sediment	6	1.74	Good
BF 500-3 Whole System	6	1.56	Good
BF 500-11 Water	4	7.62	Medium
BF 500-11 Whole System	3	6.41	Good
BF 500-14 Water	10	5.16	Good
BF 500-14 Whole System	8	5.30	Good

Table 113: Fitted parameter values and statistical assessment for metabolite dissipation at M-I level

	Parameter	Estimated value	Std error	type l error rate
BF 500-3 Sediment	M(0)	16.20	0.44	<0.001
	k	8.82E-03	1.51E-03	0.002
BF 500-3 Whole System	M(0)	20.31	0.98	<0.001
	k	7.49E-03	2.59E-03	0.022
BF 500-11 Water	M(0)	11.96	0.90	0.003
	k	2.75E-02	5.38E-03	0.018
BF 500-11 Whole System	M(0)	10.57	0.86	0.026
	k	3.06E-02	6.81E-03	0.070
BF 500-14 Water	M(0)	10.69	0.44	<0.001
	k	4.37E-02	4.15E-03	<0.001
BF 500-14 Whole System	M(0)	11.04	0.53	<0.001
	k	4.01E-02	4.22E-03	<0.001

Table 114: Modeling endpoints for metabolite dissipation evaluated at M-I level

	DT_{50} [days]
BF 500-3 Sediment	78.55
BF 500-3 Whole System	92.54
BF 500-11 Water	25.22
BF 500-11 Whole System	22.62
BF 500-14 Water	15.88
BF 500-14 Whole System	17.29

Degradation

Evaluations of the degradation kinetics of BF 500-3, BF 500-11 and BF 500-14 were carried out for the water-sediment system. In contrast to the dissipation kinetics, in which only the metabolites are considered from the point of maximum occurrence, in the evaluation of the degradation kinetics both parent and metabolite are considered using all data points from DAT = 0 onwards.

The formation and degradation of BF 500-3 in this evaluation could not be adequately reproduced by the SFO model, as the model generally overestimated the concentrations in the early stages of the simulation, but underestimated maximum occurrence concentrations. There was no justification to remove any points as experimental outliers. For the metabolite BF 500-3 the degradation rate could not be adequately determined.

For the remaining two metabolites the formation and degradation in the water-sediment system could be adequately described by the SFO kinetic model, with the t-test passed in all cases. The resulting modeling endpoints are given in Table 115.

Table 115: Modeling endpoints for metabolite system degradation evaluated at M I level

	$DegT_{50}$ [days]
BF 500-11	22.90
BF 500-14	8.21

Kinetic evaluations of an irradiated water/sediment study were carried out according to the FOCUS kinetics

recommendations [*FOCUS (2006)*] to determine modeling endpoints for pyraclostrobin. The experimental data were evaluated using single first order (SFO) kinetic models at levels P-I, P-II and M-I. In addition to the parent compound, the metabolites BF 500-3, BF 500-11 and BF 500-14 were considered.

At the M-I level dissipation kinetics were evaluated for the metabolites BF 500-11 and BF 500-14 in the water compartment and for BF 500-3 in the sediment compartment. Degradation kinetics in the complete system were evaluated for all three metabolites. The metabolite BF 500-13 could not be evaluated on the basis of the experimental data.

The endpoints for FOCUS surface water modeling according to the selection scheme given in the FOCUS Kinetics Guidance are given in Table 116 to Table 120.

Table 116: Endpoints for pyraclostrobin for FOCUS surface water modeling

FOCUS surface water Step	Endpoint	Comment
Step 1	7.22 d	System $DegT_{50}$, Level P-I
Step 2	7.5 d for water 6.48 d for sediment	Water $DegT_{50}$, Level P-II Sediment $DegT_{50}$, Level P-II
Step 3	7.5 d for water 6.48 d for sediment	Water $DegT_{50}$, Level P-II Sediment $DegT_{50}$, Level P-II

Table 117: Endpoints for metabolite BF 500-3 for FOCUS surface water modeling

FOCUS surface water Step	Endpoint	Comment
Step 1	92.54 d	System $DisT_{50}$, Level M-I
Step 2	92.54 d for water 92.54 d for sediment	System $DisT_{50}$, Level M-I System $DisT_{50}$, Level M-I

Table 118: Endpoints for metabolite BF 500-11 for FOCUS surface water modeling

FOCUS surface water Step	Endpoint	Comment
Step 1	22.62 d	System $DisT_{50}$, Level M-I
Step 2	22.62 d for water 22.62 d for sediment	System $DisT_{50}$, Level M-I System $DisT_{50}$, Level M-I

Table 119: Endpoints for metabolite BF 500-13 for FOCUS surface water modeling

FOCUS surface water Step	Endpoint	Comment
Step 1	1000 d	Conservative default value
Step 2	1000 d for water 1000 d for sediment	Conservative default value

Table 120: Endpoints for metabolite BF 500-14 for FOCUS surface water modeling

FOCUS surface water Step	Endpoint	Comment
Step 1	17.29 d	System $DisT_{50}$, Level M-I
Step 2	17.29 d for water 17.29 d for sediment	System $DisT_{50}$, Level M-I System $DisT_{50}$, Level M-I

The best-fit $DegT_{50}$ was 7.2 days in the total system (Level P-I), and the best-fit $DegT_{50}$ were 7.5 and 6.5 days in the water and sediment phase, respectively (Level P-II).

For the metabolites BF 500-11 and BF 500-14, the best-fit total system $DegT_{50}$ values were 22.6 and 17.3 days, respectively. For the metabolite BF 500-3, a system $DegT_{50}$ of 92.5 days could be determined. For the

metabolite BF 500-13, no reliable dissipation and degradation rates could be determined. Consequently, a conservative default value of 1000 days should be used for risk assessment.

Conclusion

The purpose of the kinetic evaluation was to derive modeling endpoints. However, the evaluated study *Ebert, 1999* was conducted under irradiated conditions, hence deviating from current guideline *OECD 308*. Therefore, the results of the kinetic evaluation can be seen only as supplemental information.

11.1.3.3.6 Water sediment studies – irradiated conditions

Author:	Ebert D.
Title:	Degradation of BAS 500 F in water/sediment under irradiated conditions
Date:	22.05.2012
Doc ID:	2011/1101715
Guidelines:	OECD 308, EPA 835.4300
GLP:	yes
Validity:	not acceptable, deviating from current guideline OECD 308

The degradation of ^{14}C-pyraclostrobin in aerobic water/sediment systems was investigated under irradiated conditions. Test vessels were treated separately with chlorophenyl-^{14}C- and tolyl-^{14}C-labeled pyraclostrobin with a concentration corresponding to a field application rate of about 500 g a.s. ha^{-1} when assuming overspray over a 1 m deep water body.

The test vessels were connected to an aeration system and placed in a climatic chamber (phytotron) providing a uniform day/night cycle with 13 hours light (constant light intensity of about 28 kilolux) and 11 hours dark. The temperature in the test vessels was kept in a range of about 21 – 25 °C during daylight and 18 – 20 °C during night.

Samples were taken at 0, 0.25, 1, 3, 7, 10, 14, 21, 29, 35, and 42 days after treatment (DAT).

Pyraclostrobin dissipated quickly from the water phase reaching ≤ 35% TAR already after 1 day and < 0.5 % TAR at the end of incubation.

Numerous medium polar and polar metabolites were formed in water, of which BF 500-11 (max. 5.4 % TAR), and BF 500-14 (max. 2.5% TAR) were included in the kinetic analysis. All identified metabolites decreased again during the course of the study or even disappeared completely during the last sampling intervals. Several unknown products were detected in the water during the incubation time; however, none of them ever exceeded 3.8% TAR and all appeared to be of transient nature.

In the sediment, pyraclostrobin quickly reached its maximum amounts after three days (54 - 63% TAR) and declined moderately fast to 15 - 18% TAR at the end of incubation by incorporation into the humic substance matrix.

Only low amounts of metabolites were extractable from the sediment. The des-methoxy- metabolite (BF 500-3) was formed in maximum amounts of 4 - 6% TAR and declined again towards the end of incubation to 1 – 3 % TAR. The two dimeric structures BF 500-6 (500M01) and BF 500-7 (500M02) never exceeded 3.7 and 2.3 % TAR, respectively.

The non-extractable residues reached amounts of 63 – 66 % TAR at the end of incubation, with the majority of radioactivity associated with the humic acids (max. 17 – 18 % TAR) and only a small portion associated with the fulvic acids (max. 5 – 7 % TAR).

Mineralization was detectable but overall very low (max. 3 – 4 % TAR). No other volatiles were observed.

In the dark control vessels, pyraclostrobin also disappeared quickly from the water phase, however, the degradation in the sediment (formation of bound residues) was slower compared to the irradiated incubation.

Kinetic analysis was performed following the recommendations of the FOCUS Kinetics workgroup. Pyraclostrobin degraded rather fast in the total water/sediment system with a DT_{50} of 13.5 days under irradiated conditions. It quickly dissipated from the water phase with a DT_{50} of < 0.5 days. In sediment, further degradation led to incorporation into the organic matrix (humic substances) with a DT_{50} of about 20 days. The metabolites degraded with the following half-lives: 29.1 days (BF 500-3, whole system), 9.0 days (BF 500-11, water) and 6.7 days (BF 500-14, sediment).

Overall, the results of this study showed that pyraclostrobin degraded relatively fast under irradiated as well as under dark conditions. Under sunlight-similar conditions, several photolytical breakdown products appeared in the water phase, which contributed to a faster degradation rate also in the sediment compared to dark conditions.

Material and Methods

Table 121: Test material

Pyraclostrobin (BAS 500 F)	
Chemical name:	methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3yl]oxymethyl}phenyl)-(N-methoxy)carbamate
Molecular formula:	$C_{19}H_{18}ClN_3O_4$
Molar mass:	387.82 g mol^{-1} (unlabeled)
Label 1 (chlorophenyl label)	
Label:	chlorophenyl-U-^{14}C
Batch No.:	579-4006
Specific activity of a.s.:	7.68 MBq mg^{-1}
Radiochemical purity:	99.6 %
Label 2 (tolyl label)	
Label:	tolyl-U-^{14}C
Batch No.:	566-4040
Specific activity of a.s.:	7.31 MBq mg^{-1}
Radiochemical purity:	98.7 %

Test system

A natural water and sediment system was sampled: “Berghäuser Altrhein”, a pond-like side arm of the river Rhine, south of Speyer surrounded by a forest.

The sediment was passed through a 2 mm sieve, and the water was filtered through a 0.2 mm sieve. Sieving of sediment and filling of sediment and water into the test vessels was done at the day of collecting the water/sediment system. The physico-chemical properties of the systems are summarized in Table 122.

Table 122: Characterization of the water/ sediment system

Designation **Origin**		**Berghäuser Altrhein** **Speyer, Germany**
Water		
pH		7.35
Hardness	[mmol $CaCO_3$ L^{-1}]	1.78

Designation **Origin**		**Berghäuser Altrhein** **Speyer, Germany**	
		Beginning	**End**
Dissolved organic C	[mg L^{-1}]	8.0	9.9
Total N	[mg L^{-1}]	0.68	0.81
Total P	[mg L^{-1}]	0.19	0.033
Bacteria	[cfu mL^{-1}]	6.2 x 10^3	4.0 x 10^4
Fungi	[cfu mL^{-1}]	6	2
Actinomycetes	[cfu mL^{-1}]	0	0
Sediment			
Textural class		USDA	DIN
Sand	[%]	13.3	9.3
Silt	[%]	57.3	61.3
Clay	[%]	29.4	29.4
		Silt clay loam	Loamy silt
pH (H_2O)		8.0	
pH ($CaCl_2$)		7.4	
CEC	[$cmol^+$ kg^{-1}]	34.4	
		Beginning	**End**
Organic C	[%]	5.77	6.51
Total N	[%]	0.45	0.48
Total P	[mg kg^{-1}]	470	456
Bacteria	[cfu g^{-1}]	1.4 x 10^8	4.0 x 10^7
Fungi	[cfu g^{-1}]	3.7 x 10^5	3.9 x 10^5
Actinomycetes	[cfu g^{-1}]	3.1 x 10^5	2.1 x 10^5
CEC = cation exchange capacity			

Experimental conditions

A total of 45 flasks were prepared: 15 flasks for incubation under irradiated conditions and 5 flasks for the dark control for each label. In addition, 3 flasks were prepared for system characterization at the end of the incubation and 2 flasks for continuous temperature recording.

The flasks were filled with about 130 g of wet sediment and 290 mL of water. This corresponded to a sediment layer of about 2 cm and a water layer of about 6 cm. After being filled with sediment and water, the flasks were allowed to equilibrate for 21 days under dark conditions.

Appropriate amounts (20 µL) of the respective application solutions were pipetted to the water surface to achieve a nominal amount of about 15 µg test item per test vessel. This corresponds to a field application rate of about 500 g active substance/ha assuming overspray over a 1 m deep water body. The amount of test item per test vessel is calculated for a 300 mL water volume.

The test vessels were capped air-tight and the upper 1-2 cm water layer was slightly agitated to keep the oxygen

saturation on a sufficient high level. Either one or two of the treated test vessels were connected to a volatile trapping system of two gas washing flasks containing different trapping solutions for potential ^{14}C-volatiles (ethylene glycol, 0.5 M NaOH).

Equilibration and subsequent incubation was carried out in a climatic chamber (phytotron) with temperature and light control. The test vessels were connected to a system providing a constant stream of air to be able to establish a full ^{14}C-material balance.

Incubation was conducted simulating a uniform day/night cycle with 13 hours irradiation and 11 hours dark (light intensity: 28 kilolux) in a phytotron chamber with the light being very similar to natural sunlight. The air temperature in the phytotron was kept in the range between 16 and 17.5°C, the temperature in the test vessels was kept in a range of about 21 - 25°C during daylight and 18 - 20°C during night.

The equilibration was monitored by measuring redox potential of water and sediment, temperature, O_2 content and pH of randomly selected flasks at intervals of a few days. After treatment, the same parameters were measured in each sample before workup during the irradiation phase. Dark control samples were also processed.

Sampling

Samples for the irradiated experiment were taken at 0, 0.25, 1, 3, 7, 10, 14, 21, 29, 35, and 42 days after treatment (DAT). Since two radio-labels were tested separately, they can be considered as replicates for the degradation results of the test item. Dark control samples were worked up after 14, 29 and 42 days.

Kinetic modeling

The kinetic analysis was carried out following the recommendations of the FOCUS work group on degradation kinetics [*FOCUS (2006)*] in order to derive persistence and modeling aquatic degradation endpoints.

Kinetic evaluations were performed for pyraclostrobin and three of its metabolites (BF 500-3, BF 500-11, BF 500-14) considering the different levels proposed by the FOCUS kinetics guidance. For the parent substance, the analysis at P-I level (one-compartment approach) was done for degradation in the whole system as well as the respective dissipations from the water and sediment phases of the test system. At the P-II level (two-compartment approach), the kinetic analysis considered the degradation in water and sediment taking into account the partitioning between the two phases.

For the metabolites (Level M-I), both dissipation and degradation calculations were performed.

The goodness-of-fit of the model was assessed by visual assessment as well as by means of statistical measures as recommended by the FOCUS work group on degradation kinetics.

Results and Discussion

The distribution of radioactivity in the different compartments of the water/sediment system treated with ^{14}C-labels of pyraclostrobin is presented in Table 123 and Table 124.

The material balances in the test vessels ranged between 93.6 and 98.6% of the total applied radioactivity (TAR) for the chlorophenyl-label and between 94.0 and 98.2% TAR for the tolyl-label.

The radioactivity in the water of the irradiated test vessels quickly declined with both labels to 38 - 39% TAR after one day to about 4% at the end of incubation after 42 days. The reduction of radioactivity in the water phases of the dark controls was in principle comparable, reaching 3 - 5% TAR at the end of incubation.

Corresponding to the decline of radioactivity in the water phase, the total radioactivity in the sediment quickly increased within the first 14 days to 82 and 85% TAR in the irradiated vessels, and 91 and 80% TAR in the dark controls for chlorophenyl and tolyl-label, respectively. At the end of the incubation the total amount of radioactivity increased even further to 88 - 89% TAR in the irradiated vessels and 90 - 93% TAR in the dark controls.

At the earlier sampling times, the major part of radioactivity in sediment was always extractable, but under irradiated conditions, the non-extractable part increased with time up to 63 - 66% TAR after 42 days. In the dark controls, the non-extractable radioactivity also increased, but reached only 50 - 51% TAR at the end of the incubation.

Overall, the degradation of pyraclostrobin in water/sediment systems was characterized by a very low mineralization rate. The amount of $^{14}CO_2$ never exceeded 5% TAR within 100 days. No other volatiles were detected.

Table 123: Material balance and distribution of radioactivity after application of chlorophenyl-^{14}C-pyraclostrobin to water/sediment system Berghäuser Altrhein and incubation under irradiated conditions [%TAR]

DAT	Water	Sediment							Volatiles		Material balance
	Total	ACN/ H_2O 1	ACN/ H_2O 2	ACN 1	ACN 2	Total extract-ability	NER	Total	Ethylene glycol	NaOH (CO_2)	
Irradiated											
0	94.9	1.3	0.3	0.1	0.1	1.8	0.0	1.8	n.p.	n.p.	96.8
0.25	58.7	26.7	7.7	2.9	1.0	38.4	1.5	39.9	0.0	0.0	98.6
1	37.9	38.6	11.0	4.2	1.3	55.1	3.4	58.5	0.0	0.0	96.4
3	22.2	45.2	14.2	4.0	1.9	65.2	8.7	73.9	0.1	0.2	96.4
7	14.2	39.2	12.6	5.7	2.1	59.7	20.4	80.0	0.0	0.8	95.0
10	10.2	36.4	13.5	5.2	2.3	57.5	26.9	84.3	0.0	0.4	94.9
14	10.6	31.0	8.8	6.7	3.2	49.7	32.2	82.0	0.1	1.7	94.3
21	6.5	22.7	8.5	6.5	2.9	40.7	46.2	87.0	0.0	1.8	95.3
29	6.3	19.4	7.7	6.0	2.2	35.3	49.1	84.4	0.0	2.8	93.6
35	4.4	15.1	6.1	6.0	2.9	30.1	57.5	87.6	0.1	3.5	95.6
42	4.0	12.2	4.0	5.4	3.0	24.7	62.9	87.6	0.0	4.2	95.7
Dark control											
14	6.7	42.5	9.0	9.1	5.6	66.3	24.6	90.9	0.0	0.1	97.7
29	2.9	29.6	9.8	9.2	3.2	51.7	41.5	93.3	0.0	0.4	96.6
42	2.8	23.7	7.2	8.7	3.8	43.4	49.9	93.3	0.0	0.7	96.8

ACN = acetonitrile
DAT = days after treatment
n.p. = not performed
NER = non-extractable residues
TAR = total applied radioactivity

Table 124: Material balance and distribution of radioactivity after application of tolyl-^{14}C-pyraclostrobin to water/sediment system Berghäuser Altrhein and incubation under irradiated conditions [%TAR]

DAT	Water	Sediment							Volatiles		Material balance
	Total	ACN/ H_2O 1	ACN/ H_2O 2	ACN 1	ACN 2	Total extract-ability	NER	Total	Ethylene glycol	NaOH (CO_2)	
Irradiated											
0	93.3	0.3	0.1	0.1	0.0	0.6	0.1	0.7	n.p.	n.p.	94.0
0.25	63.2	22.8	6.1	2.5	0.8	32.2	1.3	33.5	0.0	0.0	96.7
1	38.8	37.8	11.3	3.9	1.4	54.4	3.5	58.0.	0.0	0.0	96.8
3	25.5	42.6	13.2	3.7	1.7	61.2	10.5	71.7	0.0	0.1	97.3

DAT	Water	Sediment							Volatiles		Material balance
	Total	ACN/ H_2O 1	ACN/ H_2O 2	ACN 1	ACN 2	Total extract-ability	NER	Total	Ethylene glycol	NaOH (CO_2)	
7	17.2	39.2	12.2	5.5	2.1	59.0	21.8	80.9	0.0	0.2	98.2
10	11.5	34.1	13.6	4.8	2.2	54.7	30.9	85.6	0.0	0.4	97.5
14	10.8	28.7	8.6	5.4	3.1	45.8	39.1	84.9	0.0	0.4	96.0
21	7.2	24.0	8.6	6.2	2.7	41.5	46.8	88.2	0.0	1.0	96.5
29	5.5	14.9	6.1	5.7	2.2	28.8	59.8	88.6	0.0	2.0	96.1
35	5.0	13.2	5.1	5.2	2.5	26.1	62.9	89.0	0.0	2.5	96.4
42	3.8	11.7	4.3	4.8	2.5	23.4	65.9	89.3	0.0	3.4	96.5
Dark control											
14	15.5	37.0	10.2	6.1	3.6	56.8	22.8	79.7	0.0	0.3	95.5
29	8.8	26.7	8.9	7.5	2.5	45.6	39.7	85.4	0.0	1.0	95.2
42	5.3	21.7	6.9	6.9	3.0	38.5	51.4	89.9	0.0	1.7	96.9

TAR = total applied radioactivity
DAT = days after treatment
ACN = acetonitrile
NER = non-extractable residues
n.p. = not performed

Characterization and identification of residues in water and sediment extracts

An overview of active ingredient and metabolites for the water samples and sediment extracts is presented in Table 125 to Table 127Pyraclostrobin dissipated quickly from the water phase reaching ≤ 35% TAR already after 1 day and < 0.5 % TAR at the end of incubation after 42 days.

Water

Numerous medium polar and polar metabolites were formed of which BF 500-5 (max. 0.9 % TAR), BF 500-11 (max. 5.4 % TAR), BF 500-12 (max. 1.4 % TAR), BF 500-13 (max. 0.9% TAR) and BF 500-14 (max. 2.5 % TAR) could be identified by co-chromatography with reference compounds. BF 500-11, BF 500-13 and BF 500-14 were confirmed by HPLC/MS-MS analysis. All of those identified metabolites decreased again or disappeared completely during the last sampling intervals.

The des-methoxy-metabolite of pyraclostrobin (BF 500-3) could also be sporadically detected, which is explainable since the degradation route of pyraclostrobin is supposed to start with the des-methoxylation at the carbamate group. However, the appearance of this compound with 2.5% TAR in the 0 day sample of the tolyl-label treated vessel may also be attributed to the fact that it was already present as impurity in the treatment solution.

Several unknown products were detected in the water during the incubation time, however, none of them ever exceeded 3.8 % TAR, and all appeared to be of transient nature.

As expected, the photolysis breakdown products like BF 500-14, BF 500-13, and BF 500-12 were not detected in the dark controls.

Sediment

Because of the fast precipitation, pyraclostrobin reached its maximum amounts of 63 and 54 % TAR, for chlorophenyl-label and tolyl-label, respectively, at three days after treatment. Afterwards it declined moderately fast by incorporation into the organic sediment matrix, amounting to 18 and 15 % TAR at the end

of incubation.

Metabolites in the sediment extract were detected only at low amounts among which BF 500-3, BF 500-6 and BF 500-7 could be identified by co-chromatography with reference compounds and HPLC/MS-MS analysis.

The des-methoxy-metabolite (BF 500-3) was formed at maximum amounts of 4 – 6 % TAR and declined again towards the end of incubation to 1 – 3 %. The two dimeric structures BF 500-6 (500M01) and BF 500-7 (500M02) never exceeded 3.7 and 2.3 % TAR, respectively.

The extractable amount of pyraclostrobin in the sediment at the end of incubation was higher in the dark control samples than under irradiated conditions. In the time interval 14 to 42 days, the active substance decreased under dark conditions from 59 to 32 % TAR with the chlorophenyl-label and from 49 to 29 % TAR with the tolyl-label. The metabolite BF 500-3 was detected only in low amounts of <3 %, whereas BF 500-6 and BF 500-7 reached up to 7 % and 4 % TAR, respectively.

Non-extractable residues

The non-extractable (bound) residues reached high amounts of 63 and 66 % TAR during the incubation and were therefore further characterized (Table 129).

For all investigated samples, less than half of the radioactivity associated with the organic matrix in the sediment could be released by NaOH extraction. After separation of fulvic and humic acids, the majority of radioactivity stayed in the humic acids (max. 17 – 18 % TAR) and only a small portion was measured in the fulvic acids (max. 5 - 7% TAR).

Since under irradiated conditions, the formation of non-extractable residues was faster than under dark conditions, it can be concluded that the photolytical breakdown in the water phase also contributed to the overall faster degradation of pyraclostrobin in the sediment, or rather on the sediment surface. The similar distribution of the two radiolabels between the humic substance fractions showed that the two ring moieties were both readily incorporated into the humic substances in the sediment.

Table 125: Metabolite overview for water after application of chlorophenyl-^{14}C-pyraclostrobin to system Berghäuser Altrhein and incubation under irradiated conditions [%TAR]

DAT	^{14}C total	Unknown tR 7.2	Unknown tR 8.1	Unknown tR 8.7	BF 500-5 tR 51.4	BF 500-14 tR 58.8	BF 500-12 tR 69.6	BF 500-3 tR 92.9	Pyraclostrobin tR 94.4	Others*
Water – irradiated										
0	94.9	n.d.	n.d.	n.d.	0.9	n.d.	n.d.	n.d.	94.0	n.d.
0.25	58.7	n.d.	n.d.	n.d.	0.7	1.2	1.4	0.5	53.7	1.2
1	37.9	n.d.	2.5	n.d.	0.4	1.0	n.d.	n.d.	32.9	0.9
3	22.2	3.8	2.2	n.d.	n.d.	2.5	n.d.	n.d.	13.3	0.4
7	14.2	2.2	2.1	2.4	n.d.	1.7	n.d.	n.d.	5.7	n.d.
10	10.2	2.5	2.3	0.7	n.d.	1.0	n.d.	0.2	2.5	1.1
14	10.6	2.0	2.5	1.6	n.d.	1.0	n.d.	n.d.	3.6	n.d.
21	6.5	1.2	2.9	n.d.	n.d.	0.5	n.d.	0.1	1.6	0.2
29	6.3	1.8	2.6	1.1	n.d.	0.3	n.d.	n.d.	0.4	0.1
35	4.4	1.5	2.3	0.4	n.d.	n.d.	n.d.	n.d.	0.2	n.d.
42	4.0	1.1	2.1	0.3	n.d.	n.d.	n.d.	n.d.	0.4	n.d.
Water - dark control										
14	6.7	n.d.	n.d.	n.d.	1.0	n.d.	n.d.	n.d.	5.2	0.5

DAT	^{14}C total	Unknown tR 7.2	Unknown tR 8.1	Unknown tR 8.7	BF 500-5 tR 51.4	BF 500-14 tR 58.8	BF 500-12 tR 69.6	BF 500-3 tR 92.9	Pyraclostrobin tR 94.4	Others*
29	2.9	0.2	0.3	0.2	0.3	n.d.	n.d.	n.d.	1.4	0.5
42	2.8	0.4	0.6	0.1	0.3	n.d.	n.d.	n.d.	1.4	n.d.

* sum of several peaks, each single peak < 2 % TAR
tR = retention time [min], approx. value
n.d. = not detected

Table 126: Metabolite overview for sediment after application of chlorophenyl-^{14}C-pyraclostrobin to system Berghäuser Altrhein and incubation under irradiated conditions [%TAR]

DAT	^{14}C total	BF 500-3 tR 81.5	Pyraclostrobin tR 83.2	BF 500-6 tR 105.7	BF 500-7 tR 110.3	Others*
Sediment extract - irradiated						
0	1.8	n.d.	n.d.	n.d.	n.d.	n.d.
0.25	38.4	0.5	37.9	n.d.	n.d.	n.d.
1	55.1	0.8	54.2	0.1	n.d.	n.d.
3	65.2	1.4	63.0	0.5	0.2	n.d.
7	59.7	4.2	53.3	0.9	0.6	0.5
10	57.5	2.4	52.3	1.7	1.1	n.d.
14	49.7	2.2	44.0	1.9	1.0	0.7
21	40 7	2.3	33.3	2.5	1.8	0.8
29	35 3	2.0	28.1	3.1	1.8	0.3
35	30.1	1.8	21.5	3.7	1.8	1.3
42	24.7	1.0	17.5	3.6	2.2	0.4
Sediment extract- dark control						
14	66.3	0.9	58.5	3.6	2.4	0.9
29	51.7	0.9	41.1	5.9	3.1	0.7
42	43.4	0.7	31.8	7.0	3.6	0.4

* sum of several peaks, each single peak < 2% TAR
tR = retention time [min], approx. value
n.d. = not detected
0 d sediment not analyzed

Table 127: Metabolite overview for water after application of tolyl-^{14}C-pyraclostrobin to system Berghäuser Altrhein and incubation under irradiated conditions [%TAR]

DAT	^{14}C total	UK tR 7.2	UK tR 8.0	UK tR 29.2	UK tR 35.2	BF 500-13 tR 41.3	BF 500-11 tR 43.9	BF 500-14 tR 58.8	BF 500-12 tR 69.6	BF 500-3 tR 93.0	Pyraclostrobin tR 94.4	Others*
Water - irradiated												
0	93.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.5	86.5	4.4
0.25	63.2	n.d.	n.d.	n.d.	3.2	n.d.	3.0	1.9	1.0	0.5	46.7	6.9
1	38.8	n.d.	n.d.	0.7	2.7	n.d.	5.4	1.7	n.d.	1.3	22.7	4.2

DAT	^{14}C total	UK tR 7.2	UK tR 8.0	UK tR 29.2	UK tR 35.2	BF 500-13 tR 41.3	BF 500-11 tR 43.9	BF 500-14 tR 58.8	BF 500-12 tR 69.6	BF 500-3 tR 93.0	Pyraclostrobin tR 94.4	Others*
3	25.5	1.5	n.d.	2.2	1.4	n.d.	4.8	2.2	n.d.	n.d.	9.5	4.0
7	17.2	1.3	2.4	2.6	n.d.	0.6	3.5	1.6	n.d.	n.d.	3.7	1.4
10	11.5	1.4	1.4	1.4	0.5	n.d.	2.4	1.1	n.d.	n.d.	1.5	1.7
14	10.8	n.d.	1.7	1.1	n.d.	0.6	2.2	0.5	n.d.	n.d.	2.5	2.1
21	7.2	n.d.	1.0	0.6	n.d.	0.9	1.4	0.4	n.d.	0.2	1.1	1.7
29	5.5	n.d.	2.0	n.d.	n.d.	0.5	0.7	n.d.	n.d.	n.d.	0.2	2.1
35	5.0	1.6	1.8	n.d.	n.d.	0.4	0.1	n.d.	n.d.	0.3	0.4	0.3
42	3.8	1.4	1.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	0.8
Water - dark control												
14	15.5	2.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	13.4	n.d.
29	8.8	0.9	1.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	6.1	0.4
42	5.3	0.9	1.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.8	0.3

* sum of several peaks, each single peak < 2 % TAR
n.d. = not detected
tR = retention time [min], approx. value
UK = unknown

Table 128: Metabolite overview for sediment after application of tolyl-^{14}C-pyraclostrobin to system Berghäuser Altrhein and incubation under irradiated conditions [%TAR]

DAT	^{14}C total	BF 500-3 tR 81.5	Pyraclostrobin tR 83.2	BF 500-6 tR 105.7	BF 500-7 tR 110.3	Others*
Sediment extract - irradiated						
0	0.6	n.d.	n.d.	n.d.	n.d.	n.d.
0.25	32.2	1.6	30.2	n.d.	n.d.	0.4
1	54.4	3.8	49.6	n.d.	n.d.	1.0
3	61.2	4.6	54.2	0.3	0.3	1.8
7	59.0	2.5	53.8	1.1	0.7	0.9
10	54.7	0.9	53.7	n.d.	n.d.	n.d.
14	45.8	4.4	38.0	1.6	0.9	0.9
21	41.5	5.9	30.8	1.4	1.0	2.4
29	28.8	2.8	20.7	3.1	2.3	n.d.
35	26.1	2.9	17.5	2.9	1.4	1.3
42	23.4	2.8	14.5	3.2	1.9	1.1
Sediment extract – dark control						
14	56.8	2.5	49.3	2.3	1.4	1.3
29	45.6	1.8	36.4	4.5	2.5	0.5
42	38.5	1.6	28.8	4.2	2.5	1.4

* sum of several peaks, each single peak < 2 % TAR

tR = retention time [min], approx. value
n.d. = not detected
0 d sediment not analyzed

Table 129: Distribution of radioactivity between fulvic acids, humic acids, and humins after application of ^{14}C-pyraclostrobin to water/sediment systems [%TAR]

DAT	NER	Fulvic acids	Humic acids	Humins	Sum	Recovery [%]
Chlorophenyl label						
10	26.9	3.0	7.8	20.1	31.0	115.3
29	49.1	5.6	14.2	26.1	45.9	93.4
42	62.9	6.8	17.1	34.7	58.6	93.3
Tolyl label						
10	30.9	2.2	9.0	17.6	28.8	93.2
29	59.8	5.0	17.3	34.4	56.7	94.9
42	65.9	5.3	17.7	36.7	59.7	90.6

DAT = days after treatment
NER = non-extractable radioactivity
TAR = total applied radioactivity

A proposed route of degradation of pyraclostrobin in water/sediment systems is given in the figure below.

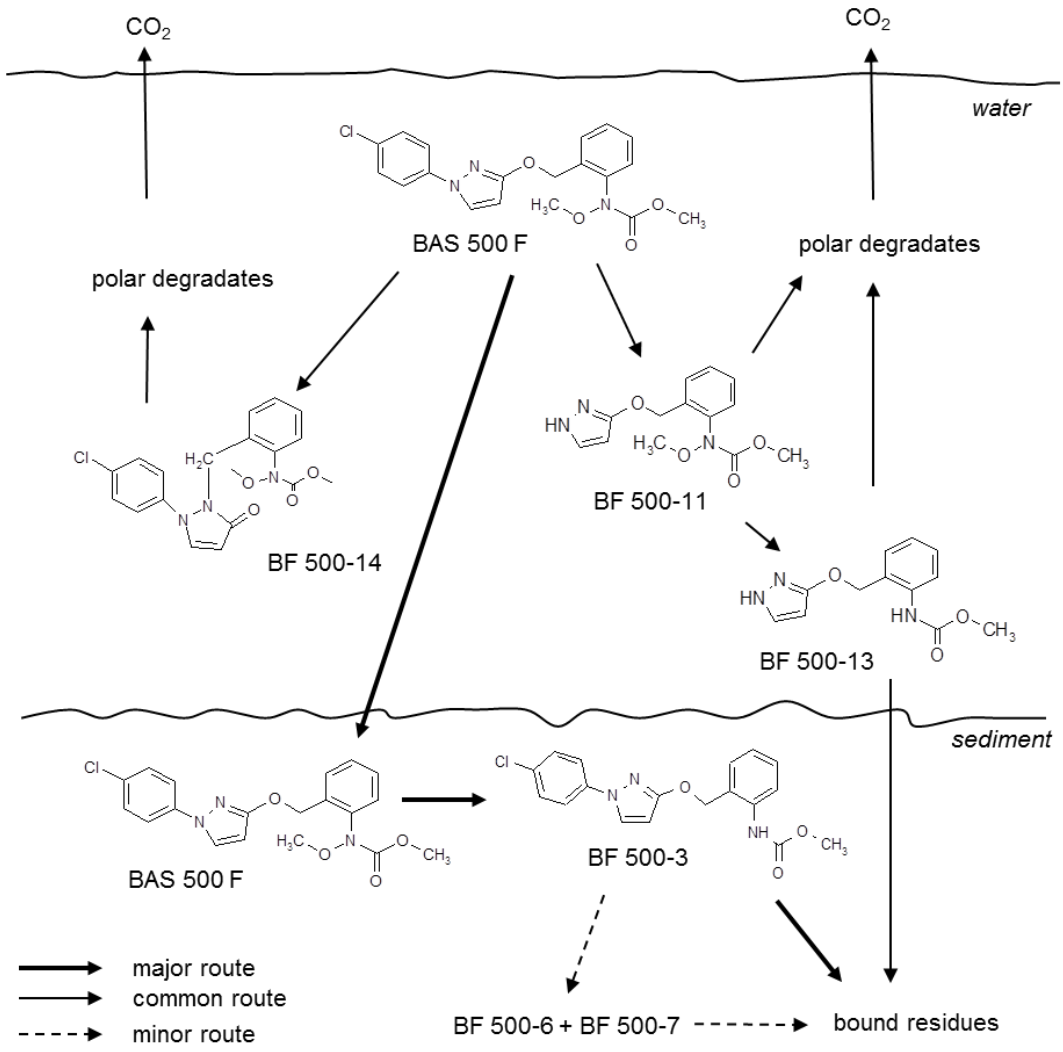


Figure 1: Proposed route of degradation of pyraclostrobin in water/sediment systems

Dissipation and degradation rates

The dissipation and degradation rates in water, sediment and the whole system could be calculated for the active substance pyraclostrobin and its metabolites BF 500-3 (sediment), BF 500-11 and BF 500-14 (both water).

The kinetics of the dissipation and degradation of BF 500-3 were established for the whole system, but using only the results of the chlorophenyl-label. The measurements for the tolyl-label in water showed a peculiarly high initial concentration of the metabolite at time zero, which was quite unusual for a metabolite forming in sediment and was not observed for the chlorophenyl-label. This fact skewed the description of the formation kinetic, and the apparition of the maximum occurrence concentration only very late toward the end of the experiment left too few measurement points to conduct the dissipation analysis. For these reasons, the measurements from the tolyl-label were discarded in the calculations of the dissipation and the degradation of BF 500-3. Furthermore, the dissipation of BF 500-3 was calculated for the whole system. While it was mainly found in sediment, the small quantities in the water phase were also considered as a worst-case perspective.

The metabolite BF 500-11 was only studied in water for both dissipation and degradation as it only appeared in this compartment. Additionally, no replicate was available as this metabolite formation implies the elimination of the molecular segment carrying the chlorophenyl-label, thus only the tolyl-label could be used to quantify it. Likewise, the metabolite BF 500-14 was studied from the tolyl-label in the water compartment

as it was not observed in sediment.

The best-fit (persistence) and modelling values for pyraclostrobin after a P-I analysis are listed in Table 130. No reliable degradation rates with a P-II analysis could be determined.

Persistence and modelling endpoints for the metabolites of pyraclostrobin are provided in Table 131 (dissipation) and Table 132 (degradation). No degradation rate for metabolite BF 500-3 could reliably be determined.

Table 130: Persistence and modeling endpoints for pyraclostrobin (P-I)

	Persistence endpoints			**Modeling endpoints**	
	Model	**DT_{50} [d]**	**DT_{90} [d]**	**Model**	**DT_{50} [d]**
Whole system [1]	HS	13.5	54.4	SFO	15.4
Water [2]	DFOP	0.3	3.9	DFOP	1.2*
Sediment [2]	SFO	20.1	66.7	SFO	20.1
[1] degradation rate [2] dissipation rate * back-calculated from DT_{90} (DT_{50} = DT_{90} / 3.32) as required by FOCUS kinetics					

Table 131: Persistence and modeling endpoints for the dissipation of the metabolites of pyraclostrobin (M-I)

	Persistence endpoints			**Modeling endpoints**	
	Model	**DT_{50} [d]**	**DT_{90} [d]**	**Model**	**DT_{50} [d]**
BF 500-3 - Whole system	not calculated			HS	29.1*
BF 500-11 – Water	SFO	9.0	29.8	SFO	9.0
BF 500-14 – Water	SFO	6.7	22.3	SFO	6.7
* back-calculated from DT_{90} (DT_{50} = DT_{90} / 3.32) as required by FOCUS kinetics					

Table 132: Persistence and modeling endpoints for the metabolites degradation (M-I)

	Persistence endpoints			**Modeling endpoints**	
	Model	**$DegT_{50}$**	**$DegT_{90}$**	**Model**	**DT_{50}**
BF 500-11 – Water	SFO	6.6	22	SFO	6.6
BF 500-14 – Water	SFO	6.9	23	SFO	6.9

Overall, it can be concluded that pyraclostrobin degrades rather fast in the total water/sediment system with a best-fit $DegT_{50}$ of 13.5 days under irradiated conditions. It quickly dissipates from the water phase with a DT_{50} of < 0.5 days. In sediment, further degradation leads to incorporation into the organic matrix (humic substances) with a DT_{50} of about 20 days.

Conclusion

The water/sediment study conducted under light conditions is helpful to understand the influence of light on the degradation and distribution of pyraclostrobin in aquatic systems. The entire evaluation of the active substance will be however done on the base of the study conducted in the dark, according to the current guideline.

11.1.3.4 Photochemical degradation

Author:	Scharf J.
Title:	Determination of the absorption coefficients of BAS 500 F
Date:	1998

Doc ID:	1998/10257
Guidelines:	BBA IV, 6-1, OECD Draft Test Guideline "Phototransformation of Chemicals in Water" Part A
GLP:	yes
Validity:	Acceptable

Author:	Scharf J.
Title:	Aqueous photolysis of BAS 500 F
Date:	14.10.1999
Doc ID:	1999/11286
Guidelines:	FAO Revised Guidelines on Environmental Criteria for the Registration of Pesticides, Revision 3, US-EPA, Subdivision N, 161-2
GLP:	yes
Validity:	acceptable

Material and Methods

The direct photolysis was performed with both labels of the active substance. The study was performed in sterile aqueous buffer solution at pH 5 (acetate buffer) and 22 ± 1 °C. The concentration of the active substance was about 0.5 mg/L.

Results and Discussion

During direct photolysis, a very fast degradation of the active substance was observed. A large number of degradation and rearrangement products occured, only some of them being stable under simulated environmental conditions. The molar mass and/or structure of 33 metabolites could be determined. Five of the metabolites BF 500-11, BF 500-13, BF 500-14, BF 500-15 and 500M58 occured once or several times with amounts >10 % TAR.

Mineralisation after 25 days was 22 % TAR (^{14}C-chlorophenyl-labelled) and 4 % TAR (^{14}C-tolyl-labelled). In the dark control, no degradation was observed. The results are summarised in Table 133 and Table 134.

Table 133: Recovery of radioactivity in % and distribution of metabolites after application of ^{14}C-chlorophenyl-labelled-pyraclostrobin during aqueous photolysis

time after treatment	**% TAR**							
	pyraclostrobin	**BF500-14 (500M76)**	**BF 500-15 (500M78)**	**500M58**	**CO_2**	**sum of minor, identified peaks***	**sum of others****	**sum**
0 h	94.1				n.m.	5.9	0.0	100.0
3 h	10.4	20.7	6.2	13.2	0.1	43.5	6.0	100.1
6 h	2.4	12.8	15.6	22.7	0.1	35.6	15.3	104.5
9 h	0.6	11.8	17.8	21.2	0.3	33.2	13.9	98.8
1 d	0.5	5.6	26.6	23.4	1.8	22.5	20.4	100.8
3 d	0.0	4.0	25.3	19.5	6.0	19.2	26.2	100.2
6 d	0.0	4.5	24.2	19.0	13.1	18.0	20.4	99.1
10 d	1.3	0.0	3.0	2.2	13.2	14.6	59.8	94.2

time after treatment	% TAR							
	pyraclostrobin	BF500-14 (500M76)	BF 500-15 (500M78)	500M58	CO_2	sum of minor, identified peaks*	sum of others**	sum
15 d	0.0	0.0	7.9	0.3	15.2	17.5	49.9	90.9
18 d	0.0	0.0	8.5	6.3	18.0	12.0	51.6	96.4
21 d	0.0	0.0	8.0	1.1	21.7	16.8	50.9	98.5
25 d	0.0	0.0	5.2	3.7	21.9	10.5	55.8	97.1

n.m.: not measured
* each peak (identified) <10% TAR at any sampling time
** each peak (not identified) < 10% TAR at any sampling time

Table 134: Recovery of radioactivity in % and distribution of metabolites after application of ^{14}C-tolyl-labelled-pyraclostrobin during aqueous photolysis

time after treatment	% TAR								
	pyraclostrobin	BF500-11 (500M60)	BF500-13 (500M62)	BF500-14 (500M76)	500M58	CO_2	sum of minor, identified peaks*	sum of others**	sum
0 h	100.0					n.m.			100.0
3 h	39.7	12.5	3.5	14.1	4.4	0.0	23.4	0.4	98.0
6 h	3.8	22.8	12.3	14.8	12.8	0.0	29.4	2.5	98.5
9 h	4.7	21.7	12.1	13.4	12.9	0.0	29.0	3.3	96.9
1 d	0.0	27.6	13.9	3.7	20.3	0.1	27.3	2.4	95.2
3 d	0.0	27.9	14.6	3.3	17.5	0.3	28.1	1.9	93.5
6 d	0.0	31.1	16.8	1.7	11.7	1.3	29.3	5.2	97.0
10 d	0.0	38.3	11.7	0.6	4.7	3.0	32.7	8.1	99.1
15 d	0.0	39.6	12.9	1.5	9.0	1.6	25.1	7.2	96.9
18 d	0.0	38.3	9.5	0.4	2.7	3.7	24.5	20.1	99.0
21 d	0.0	44.5	3.9	0.0	3.0	4.5	24.7	16.2	96.8
25 d	0.0	37.4	8.0	0.0	2.8	3.7	27.6	19.1	98.6

n.m.: not measured
* each peak (identified) <10% TAR at any sampling time
** each peak (not identified) < 10% TAR at any sampling time

The quantum yield of pyraclostrobin was estimated to be 2.17. The calculated half-lives of the active substance pyraclostrobin and its main metabolites are shown in Table 135.

Table 135: Calculated half-lives of pyraclostrobin and its major metabolites during aqueous photolysis (continuous irradiation)

substance	half-life [d]		
	chlorophenyl label	tolyl label	mean value
pyraclostrobin		0.08	0.06
BF 500-14	0.04	0.34	0.28
500M58	0.22	10.14	8.64
BF 500-15	7.14		-

substance	half-life [d]		
	chlorophenyl label	tolyl label	mean value
BF 500-11	4.62	-*	
BF 500-13	0.04	30.67	-

* no calculation possible

The proposed degradation pathway for pyraclostrobin during aqueous photolysis is shown in Figure 2.

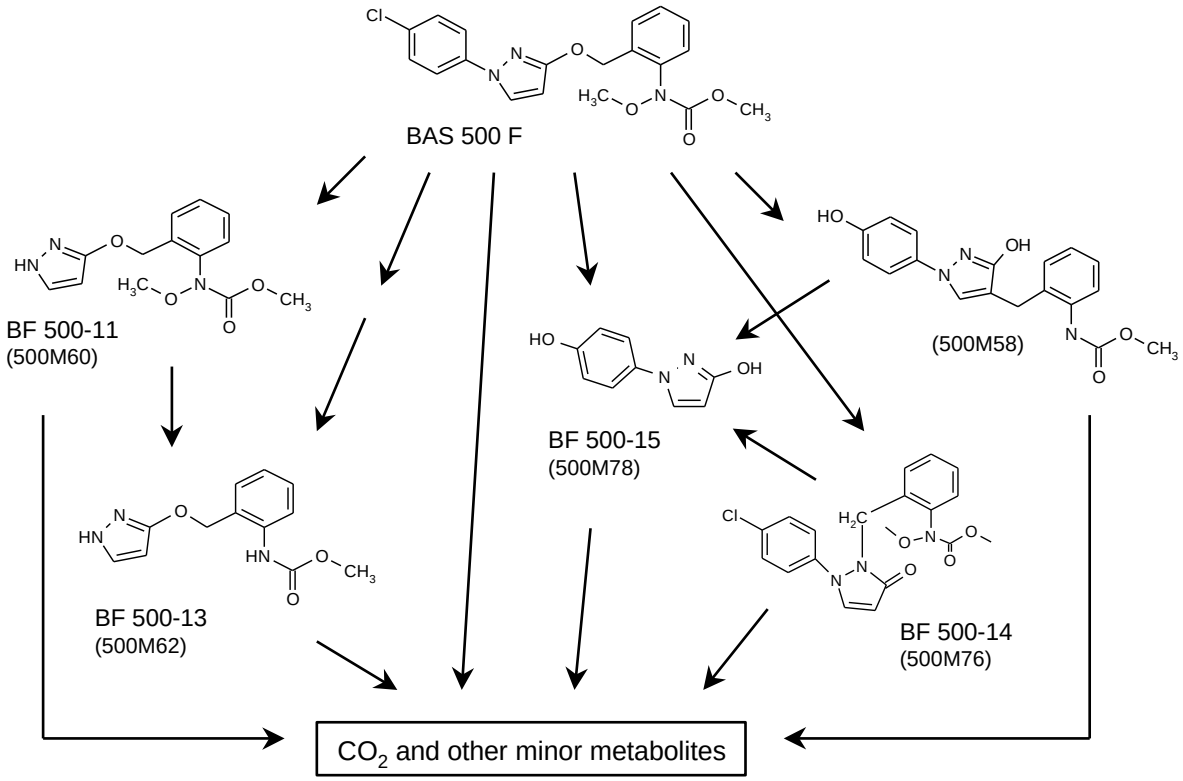


Figure 2: Proposed route of degradation of pyraclostrobin during aqueous photolysis

Conclusion

The resulting mean DT_{50} value for pyraclostrobin was 0.06 d under study conditions. In total, 5 photodegradation products (BF 500-11, BF 500-13, BF 500-14, BF 500-15 and 500M58) reached amounts of > 10% TAR.

11.2 Environmental transformation of metals or inorganic metals compounds

Not applicable.

11.2.1 Summary of data/information on environmental transformation

Not applicable.

11.3 Environmental fate and other relevant information

Not applicable.

11.4 Bioaccumulation

All the information on bioaccumulation are taken from the RAR and list of endpoints for pyraclostrobin, January 2020.

Since pyraclostrobin has a logP = 3.99, the potential for bioaccumulation was tested experimentally, resulting in a maximum whole fish BCF of 776. Hence, pyraclostrobin does fulfil the criteria for bioaccumulation.

Table 136: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
OECD 305, EPA 165-4	Lipid-normalised ^{14}C-BCF was 712 for chlorophenyl-label and 776 for tolyl-label. High difference in BCF between edibles (232-262) and inedibles (1169-1221).	Key study Reliability: 1	1999/17015: 1999
OECD 117 (HPLC)	The mean log P_{OW} was 3.99 and the corresponding P_{OW} was 9772. Effect of pH was not investigated since there is no dissociation in water.	acceptable	CHE2000-465: Tuerk, 1996

11.4.1 Estimated bioaccumulation

No data available

11.4.2 Measured partition coefficient and bioaccumulation test data

11.4.2.1

Author:	Anonymous.
Title:	Bioaccumulation and metabolism of (14C)-BAS 500 F in bluegill sunfish
Date:	01.10.1999
Doc ID:	Report number 17015; Reg Doc BASF 99/11348
Guidelines:	OECD 305, EPA 165-4
GLP:	yes
Validity:	acceptable
Previous evaluation:	In initial DAR (2001)

Material and Methods

Test item:	[Tolyl-U-14C]-BAS 500 F, radiochemical purity > 97 %
Test species:	Bluegill sunfish (*Lepomis macrochirus*)
Test design:	The accumulation of (^{14}C)BAS 500 F in bluegill sunfish study consisted of a 37-day uptake phase and a 14-day depuration phase for the chlorphenyl label and a 21-day phase

	for the tolyl label. The ^{14}C-1abelled test material was delivered continuously via a flow-through diluter system.
Endpoints:	Water and tissue residue concentrations, bioconcentration factors (BCFs), and elimination half-life.
Test concentrations:	300 ng a. i./L (nominal concentration).
Test conditions:	Three glass tanks (57 x 40 x 37 cm), each with a 60 liter capacity, were used in the study. One tank was used as control and two were used for (^{14}C)BAS 500 F exposure. N,N-dimethyl formamide was used as solvent. A random numbers table was used to assign the test organisms to appropriate test chambers. At the beginning of the accumulation study, 150 bluegill sunfish of 0.1 g each were transferred to the 16 tanks. These fish were used for sampling during the subsequent uptake and depuration phases of the accumulation study. Temperature: 20.0 – 24.0 °C (single occasions with a temperature of 19.3-19.7 °C, without influence of the study); pH 7.0-7.8; oxygen saturation > 80 %; TOC content did not exceed 2 mg/L; photoperiod: 16 h light : 8 h dark; feeding: Fish were fed daily with Salmon Fry Diet, excess food was removed after 30-60 minutes.
Analytics:	Each group of tissue samples (whole body, edible, and non-edible) was separately pooled and homogenized. HPLC was used for analyses.
Statistics:	Nonlinear regression analysis (non-linear parameter estimation NLIN of SAS version 6.07) was used to estimate the parameters k_1 and k_2. Results for concentrations of total radioactivity in each tissue and whole fish were analysed by using ANOVA, pairwise comparisons between sample points were made by Student`s t-est.

Results and Discussion

Lipid content of fish was 4.74 %.

Chlorophenyl label:

During the uptake phase the actual concentration of total radioactivity in water was in the range of 263 - 344 ng/L with a mean concentration of 305 ng/l. Water sampled at the first day of depuration contained 14% of the nominal concentration used in the uptake phase and thereafter the concentration dropped to levels below the limit of determination. The only radioactive component in water was unchanged BAS 500 F.

Mean concentrations of radioactivity in the total fish reached an apparent steady state of 184 – 235 ng/g after 4 days of exposure. Mean concentrations of radioactivity in the fillet increased from 47 ng/g on Day 1 of the exposure period to a plateau of 66 – 71 ng/g. Mean concentrations of radioactivity in the inedible fraction (viscera) increased from 180 ng/g on Day 1 to a plateau of 314 - 404 ng/g during Days 4 - 35 of the exposure period.

The bioconcentration factors and kinetic parameters based on total radioactivity concentrations were derived from Non-Linear-Regression Analysis using a 2 Compartment Model. Kinetic parameters are summarized in Table 137. The depuration half-life in whole fish was 0.9 day. Accordingly, the time to reach 90% depuration is 3.0 days. The bio-concentration factor calculated directly from the ratio of the 14C-concentrations in water and tissue fractions (mean of Days 4 – 35) was 673 for whole fish, 232 for edible tissues and 1169 for viscera (see Table 138). The values were in good accordance with those obtained by kinetic modelling.

The BCF values for unchanged BAS 500 F were 379 for whole fish, 191 for edibles and 574 for inedibles (see Table 139).

Tolyl label:

During the uptake phase the actual concentration of total radioactivity in water was in the range of 278 - 336 ng/l with a mean concentration of 300 ng/l. Water sampled at the first day of depuration contained 22% of the nominal concentration used in the uptake phase and thereafter the concentration dropped to levels below the

limit of determination. The only radioactive component in water was unchanged BAS 500 F.

Mean concentrations of radioactivity in the total fish reached an apparent steady state of 192 - 243 ng/g after 4 days of exposure. Mean concentrations of radioactivity in the fillet increased from 70 ng/g on Day 1 of the exposure period to a plateau of 74 – 84 ng/g. Mean concentrations of radioactivity in the inedible fraction (viscera) increased from 197 ng/g on Day 1 to a plateau of 331 - 425 ng/g during Days 4 - 35 of the exposure period.

The bioconcentration factors and kinetic parameters based on total radioactivity concentrations were derived from Non-Linear-Regression Analysis using a 2 Compartment Model. Kinetic parameters are summarized in Table 137). The depuration half-life in whole fish was 0.9 days. Accordingly, the time to reach 90% depuration is 2.8 days. The bio-concentration factor calculated directly from the ratio of the 14C-concentrations in water and tissue fractions (mean of Days 4 – 35) was 719 for whole fish, 262 for edible tissues and 1221 for viscera (see Table 138). The values were in good accordance with those obtained by kinetic modelling.

The BCF values for unchanged BAS 500 F were 507 for whole fish, 178 for edibles and 853 for inedibles (see Table 139).

Nature of the radioactive residues

The nature of the residues in fish fractions from the steady-state period (28 days of exposure) is summarized in Table 140. It appeared that pyraclostrobin was initially metabolized to form metabolite 500M08 and 500M45 (for chemical structures see Table 141). In addition, cleavage of the ether link between the pyrazol and the tolyl rings led to separation of the molecule into 500M04 and unidentified components. In total, five unknown components were observed. However, the major component in all tissue fractions was unchanged pyraclostrobin which comprised 39% - 74 % of the Total Radioactive Residue.

Table 137: Kinetic parameters

Parameter	**Tissue fraction**					
	Chlorophenyl label			**Tolyl label**		
	Edibles	**Viscera**	**Whole fish**	**Edibles**	**Viscera**	**Whole fish**
Uptake rate constant k_1 [$ml^{-1} \cdot g^{-1} \cdot days^{-1}$]	236	833	514	243	994	598
Depuration rate constant k_2 [$days^{-1}$]	1.020	0.711	0.762	0.901	0.797	0.812
Depuration half-life [days]	0.7	1.0	0.9	0.8	0.9	0.9
Time to reach 90% depuration t_{90} [days]	2.3	3.2	3.0	2.6	2.9	2.8
Bioconcentration factor (BCF) k_1/k_2	232	1171	675	269	1246	736

Table 138: Bioconcentration factors calculated directly from the ratio of total radioactivity concentrations in water and tissues

Uptake phase Day No.	**Tissue fraction**					
	Chlorophenyl label			**Tolyl label**		
	Edibles	**Viscera**	**Whole Fish**	**Edibles**	**Viscera**	**Whole Fish**
1	177	677	421	248	699	443
2	212	956	566	199	1135	666
4	262	1066	623	233	1041	616
7	206	981	575	274	1065	625
14	223	1052	608	261	1252	732
21	217	1142	650	252	1123	661
28	259	1474	814	273	1417	810

Uptake phase Day No.	Tissue fraction					
	Chlorophenyl label			Tolyl label		
	Edibles	Viscera	Whole Fish	Edibles	Viscera	Whole Fish
31	230	1288	761	270	1288	782
35	228	1179	683	270	1358	805
Mean Days 4 – 35 (plateau)	232	1169	673	262	1221	719

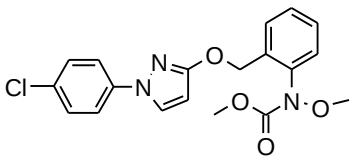
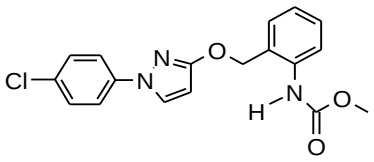
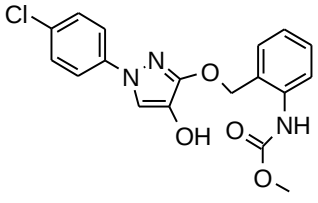
Table 139: Bioconcentration factors for unchanged parent compound calculated from the ratio of the pyraclostrobin concentrations in tissues and water

Label	BCF		
	Edibles	Inedibles	Whole Fish
Chlorophenyl label	191	574	379
Tolyl label	178	853	507

Table 140: Summary of identified metabolites in fish tissues sampled at Day 28. Values in ng/kg tissue and % of total tissue radioactivity (in parenthesis)

Metabolite	Chlorophenyl label		Tolyl label	
	Edibles	Inedibles	Edibles	Inedibles
BAS 500 F	52 (73.7)	156 (38.6)	51 (62.8)	244 (57.4)
500M07	7 (9.4)	29 (7.3)	5 (6.5)	14 (3.4)
500M08	3 (3.6)	18 (4.5)	2 (2.5)	-
500M45	-	-	3 (4.1)	-
500M04	1 (2.0)	36 (9.0)	-	-

Table 141: Structures of identified metabolites

Metabolite	Structure
BAS 500 F	
500M07 (BF 500-3)	
500M08	

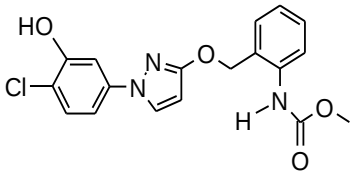
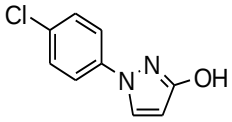
Metabolite	Structure
500M45	
500M04 (BF 500-5)	

Table 142: Validity criteria according to OECD TG 305 (2012)

Validity criteria according to OECD TG 305 (2012)	Obtained in this study
The water temperature variation is less than ± 2°C, because large deviations can affect biological parameters relevant for uptake and depuration as well as cause stress to animals. (recommended range for bluegill sunfish according to annex 3: 20 – 25°C)	20.0 – 24.0°C * variation > 2°C (over whole study period)
The concentration of dissolved oxygen does not fall below 60% saturation.	> 60% (6.4 – 9.6 mg/L)
The concentration of the test substance in the chambers is maintained within ± 20% of the mean of the measured values during the uptake phase.	Concentration of a.s. in tank water: 303 – 339 ng/L (101% – 113%).
The concentration of the test substance is below its limit of solubility in water, taking into account the effect that the test water may have on effective solubility (For multi-constituent substances such as UVCBs, the water solubility of each relevant component should be considered to determine the appropriate exposure concentrations.).	Yes (test concentration: 300 ng/L; solubility in water: 1.9 ± 0.17 mg/L at 20 C in deionized water (pH of 5.8)
The mortality or other adverse effects/disease in both control and treated fish is less than 10% at the end of the test; where the test is extended over several weeks or months, death or other adverse effects in both sets of fish should be less than 5% per month and not exceed 30% in all. Significant differences in average growth between the test and the control groups of sampled fish could be an indication of a toxic effect of the test chemical.	During exposure: 0 – 2%

Failure in terms of meeting one validity criterion/recommendation is marked in **bold.**
* Single occasion with a temperature of 19.3-19.7 °C, without influence on the study outcome

Conclusions

After exposure of bluegill sunfish to pyraclostrobin at a nominal exposure level of 300 ng/l, apparent steady state was reached after 2 - 4 days. After termination of the exposure, radioactivity levels in fish tissues decreased rapidly with a half-life of ca. 0.7 – 1.0 days. Bioconcentration factors based on total radioactivity were relatively low in edibles (232 - 262) and relatively high in inedibles (1169 - 1221). For unchanged parent compound, the BCF values were considerably lower in all tissues. This is an indication for an intensive metabolic clearance of pyraclostrobin. Only minor differences were observed between the two labelled forms of the test compound with regard to the kinetic parameters.

The bioconcentration factor (BCF) for whole fish normalized for a lipid content of 5 % was 712 for the chlorphenyl label and 776 for the tolyl label.

The minor deviation from the recommendations for the variation of water temperatures are of no relevance for the overall outcome of the study. All other validity criteria were met. The study is valid and reliable. It is relevant for classification purposes.

11.5 Acute aquatic hazard

All the information on acute toxicity are taken from the RAR and list of endpoints for pyraclostrobin, January 2020.

Table 143: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
EPA 850.1075, 72-1	*Oncorhynchus mykiss*	Pyraclostrobin (99 %)	LC_{50} (96 h) = 0.00616 mg a.s./L (mean measured) – static system	Key study Reliability: 1	12F0494/965180: 1999
EPA 72-1, EEC 92/69, OECD 203	*Lepomis macrochirus*	Pyraclostrobin (97.1 %)	LC_{50} (96 h) between 0.0196 and 0.0335 mg a.s./L (mean measured) – static system	Reliability: 2 Fish were considerably larger than recommended in the guideline	14F0494/965179: 1998
EPA 72-1, EEC 92/69, OECD 203	*Cyprinus carpio*	Pyraclostrobin (97.1 %)	LC_{50} (96 h) between 0.0121 and 0.0258 mg a.s./L (mean measured) – static system	Reliability: 2 Fish were considerably larger than recommended in the guideline	11F0494/965178: 1998
EPA 72-1(c),EPA 850.1075	*Oncorhynchus mykiss*	Pyraclostrobin (97.1 %)	LC_{50} (96 h) = 0.0062 mg a.s./L (mean measured) – flow-through	Reliability: 1	1946-BA: 2000
EPA 72-1(c), EPA 850.1075	*Cyprinodon variegatus*	Pyraclostrobin (97.1 %)	LC_{50} (96 h) = 0.0769 mg a.s./L (mean measured) – flow-through	Reliability: 1	1317-BA: 2000
EPA 72-1(c), EPA 850.1075	*Lepomis macrochirus*	Pyraclostrobin (97.1 %)	LC_{50} (96 h) = 0.0114 mg a.s./L (mean measured) – flow-through	Reliability: 1	1947-BA: 2000
OECD 203, US EPA 850.1075, EPA 72-1	*Pimephales promelas*	Pyraclostrobin (99 %)	LC_{50} (96 h) = 0.0146 mg a.s./L (mean measured) – flow-through	Reliability: 2 Fish were considerably larger than recommended in the guideline	18F0494/96E001: 2014
OECD 202, EEC 92/69	*Daphnia magna*	Pyraclostrobin (97.1 %)	EC_{50} (48 h) = 0.0157 mg a.s./L (nominal) – static system	Reliability: 1	35806: Dohmen, 1999

Method	Species	Test material	Results[1]	Remarks	Reference
EPA 850.1035, EPA 72-3(b)	*Americamysis bahia*	Pyraclostrobin (97.1 %)	LC_{50} (96 h) = 0.00416 mg a.s./L (mean measured) – flow-through	Key study Reliability: 1	1318-BA: Boeri, 2000
EPA 850.1025, EPA 72-3(c)	*Crassostrea vriginica*	Pyraclostrobin (97.1 %)	EC50 (96 h) = 0.0125 mg a.s./L (mean measured) – flow-through	Reliability: 1	1319-BA: Boeri, 2000
EPA 850.1740	*Leptocheirus plumosus*	Pyraclostrobin (99 %) + 14C-radiolabeled pyraclostrobin (99.4 %)	LC_{50} (10 d) = 4.412 mg a.s./kg dw (mean measured) – static system	Reliability: 1	68249: Gaertner, 2013
EPA 850.1735, OECD 219	*Hyalella azteca*	Pyraclostrobin (99 %)	LC_{50} (10 d) > 0.019 mg a.s./L (mean measured) – static system	Reliability: 1	2017/7018168: Staggs, 2018
OECD 201	*Pseudokirchneriella subcapitata*	Pyraclostrobin (97.1 %)	E_rC_{50} (96 h) > 0.843 mg a.s./L E_bC_{50} (96 h) = 0.152 mg a.s./L* (mean measured) – static system	Reliability: 1	35803: Dohmen, 1999 2009/1037148: Hoffmann, 2009 – additional calculation
EPA 123-2, EPA 850.5400	*Navicula pelliculosa*	Pyraclostrobin (97.1 %)	E_rC_{50} (120 h) > 0.0184 mg a.s./L (initial measured) E_rC_{50} (72 h) = 0.011 mg a.s./L (mean measured) – static system	Key study Reliability: 2	1321-BA: Boeri, 2000
EPA 123-2, EPA 850.5400	*Anabaena flos-aquae*	Pyraclostrobin (97.1 %)	E_rC_{50} (120 h) > 1.78 mg a.s./L E_rC_{50} (72 h) = 1.41 mg a.s./L (mean measured) – static system	Reliability: 3 Validity criteria not met	1222-BA: Boeri, 2000
EPA 123-2, EPA 850.5400	*Skeletonema costatum*	Pyraclostrobin (97.1 %)	E_rC_{50} (96 h) > 0.07 mg a.s./L E_bC_{50} (96 h) = 0.007 mg a.s./L* (mean measured) – static system	Reliability: 2 Reliability: 2	1223-BA: Boeri, 2000
OECD 201 (2011)	*Ankistrodesmus bibraianus*	Pyraclostrobin (99.9 %)	E_rC_{50} (72 h) = 0.221 mg a.s./L (nominal) – static system	Reliability: 1	2018/1099618: Backfisch and Englert, 2018

Method	Species	Test material	Results[1]	Remarks	Reference
OECD 201 (2011)	*Navicula pelliculosa*	Pyraclostrobin 99.9 %)	ErC_{50} (72 h) > 1.35 mg a.s./L (mean measured) – static system	Reliability: 1	2018/1194277: Eckenstein, 2018
EPA 123-2, EPA 850.4400 OECD 221	*Lemna gibba*	Pyraclostrobin (97.1 %)	E_rC_{50}/E_yC_{50} (14 d) > 1.077 mg a.s./L* (mean measured) – static system	Reliability: 1	1324-BA: Boeri, 2000 Combined with addendum study 2019/2036269: Unknown, 2019

[1] Indicate if the results are based on the measured or on the nominal concentration
* The E_bC_{50}/E_yC_{50} is given for completeness but is not considered for the CLH proposal. According to EU Regulation 1272/2008, classification should be based on E_rC_{50} values.

11.5.1 Acute (short-term) toxicity to fish

11.5.1.1 Acute toxicity to fish – *Oncorhynchus mykiss*

Author:	Anonymous
Title:	BAS 500 F - Acute toxicity study on the rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792) in a static system (96 hours)
Date:	23.09.1999
Doc ID:	12F0494/965180; BASF DocID 1999/11414
Guidelines:	EPA 850.1075, 72-1
GLP:	Yes
Validity:	Acceptable
Previous evaluation:	In initial DAR (2001)

Material and Methods

Test item:	BAS 500 F (96/494-3, batch no. CP029053); purity: 99.0 %
Test species:	Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792), mean body length 6.25 (5.9 – 6.9) cm; mean body weight 2.15 (1.7 – 3.1) g.
Test design:	Static system (96 hours); 10 fish per aquarium (loading about 0.2 g fish/L) and per concentration. Six test item concentrations and a negative (water) control; 1 replicates per treatment and control; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and after 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Negative control, 0.00316, 0.00464, 0.00681, 0.01, 0.0147 and 0.0215 mg as/L (nominal).
Test conditions:	Glass aquaria (80 x 35 x 46 cm), test volume: 100 L in a temperature-controlled room; temperature: 12 - 13 °C; pH 8.0 - 8.4; lowest dissolved oxygen concentration at test end 8.5 mg/L; photoperiod: 16 h light : 8 h dark; no feeding.
Analytics:	HPLC method CP 314.
Statistics:	No details presented in study report.

Results and Discussion

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration one hour after test start and, except for the highest concentration at the end of the test. The analyzed contents of pyraclostrobin ranged from 75.1% to 98.4% of nominal at test initiation and from 47.2% to 62.4% of nominal at test termination. The analytical results are presented in Table 144.

Table 144: Analytical results

Nominal concentration (mg/L)	**Measured concentration (mg/L) 1 h**	**Recovery (%)**	**Measured concentration (mg/L) 96 h**	**Recovery (%)**
control	n.d.	-	n.d.	-
0.00316	0.00311	98.4	0.00193	61.1
0.00464	0.00401	86.4	0.00254	54.7
0.00681	0.00531	78.0	0.00375	55.1
0.01	0.00759	75.9	0.00472	47.2
0.0147	0.01104	75.1	0.00918	62.4
0.0215	0.01730	80.5	*	-

n.d. = not detectable
* not measured as all fish died within first 24 hours

Biological results: BAS 500 F caused mortality to the rainbow trout at concentrations ≥ 0.01 mg as/L (nominal). Behavioural symptoms such as apathy, convulsions, narcotic-like state and swimming near the bottom were monitored in the 0.00681 mg as/L concentration (nominal) and above (see Table 145).

Table 145: Acute toxicity (96 h) of pyraclostrobin to rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg a.s./L] (nominal)	**Control**	**0.00316**	**0.00464**	**0.00681**	**0.0100**	**0.0147**	**0.0215**
Concentration [mg a.s./L] (mean measured)	--	0.00252	0.00328	0.00453	0.00616	0.01011	-
Mortality [%] (96 h)	0	0	0	0	50	100	100 (after 24 h)
Symptoms (after 96 h) *	none	none	none	D (1)	D (1), A(1)	-	-
Endpoints [mg pyraclostrobin/L] (mean measured)							
LC_{50} (96 h)	0.00616						
NOEC (96 h survival)	0.00453						
NOEC (96 h sublethal effects)	0.00328						

* D = swimming near bottom, A = apathy, number in bracket = number of affected fish

Validity criteria according to OECD TG 203 (1992)	**Obtained in this study**
The mortality in the control(s) should not exceed 10 % (or one if less than ten are used) at the end of the test	No fish died until test end
Constant conditions should be maintained as far as possible throughout the test and if necessary, semi-static or flow-through procedures should be used.	Remained constant
The dissolved oxygen concentration must have been at least 60 % of the air saturation value throughout the test.	> 60 % (8.5 – 10.3 mg/L)

There must be evidence, that the concentration of the substance being tested has been satisfactorily maintained, and preferably it should be at least 80% of the nominal concentration throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.	75.1 - 98.4% at test initiation and 47.2 - 62.4% at test end; therefore, results are based on mean measured concentrations

Conclusions

In a static acute toxicity study with rainbow trout the LC_{50} (96 h) of pyraclostrobin was 0.00616 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.0045 mg a.s./L (mean measured).

It is noted that the fish size was slightly greater than recommended in the OECD TG 203 (1992); *i.e.* average total length was 6.25 cm (5.9 – 6.9 cm; recommended: 5.0 ± 1.0 cm). The loading recommendation of the guideline was still followed. In the frame of the risk assessment it has to be considered that there were other fish acute studies with a clearly higher deviation of the size. As the fish size presents a reflection of the age and the age of the fish can have an impact on sensitivity this point should be taken into account for risk assessment. Regarding the fish size, the deviation from recommended size in this certain case is rather small.

The study can still be considered as valid and reliable. It is relevant for classification purposes.

11.5.1.2 Acute toxicity to fish – *Lepomis macrochirus*

Author:	Anonymous
Title:	BAS 500 F - Acute toxicity study on the bluegill (*Lepomis macrochirus* RAF.) in a static system (96 hours)
Date:	24.08.1998
Doc ID:	14F0494/965179; Reg.Doc# BASF 98/10951
Guidelines:	EPA 72-1, EEC 92/69, OECD 203
GLP:	Yes
Validity:	Acceptable
Previous evaluation:	In initial DAR (2001)

Material and Methods

Test item:	BAS 500 F (96/494-1, batch no. LJ 27882/191/C); purity: 97.1 %
Test species:	Bluegill (*Lepomis macrochirus* Raf.), mean body length 6.31 (5.5 – 7.0) cm; mean body weight 4.02 (2.5 – 5.4) g.
Test design:	Static system (96 hours); 10 fish per aquarium (loading about 0.4 g fish/L) and per concentration. Seven test item concentrations, a solvent control and a negative (water) control; 1 replicates per treatment and control; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and after 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Control, solvent control acetone, 0.00464, 0.00681, 0.01, 0.0147, 0.0215, 0.0316, 0.0464 mg as/L (nominal), stock solution in acetone.
Test conditions:	Glass aquaria (80 x 35 x 46 cm), test volume: 100 L in a temperature-controlled room; temperature: 21 - 23 °C; pH 7.0 – 7.9; lowest dissolved oxygen concentration at test end 4.0 mg/L; photoperiod: 16 h light : 8 h dark; no feeding.

Analytics:	HPLC
Statistics:	No details presented in study report.

Results and Discussion

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration one hour after test start and at the end of the test. The analyzed contents of pyraclostrobin ranged from 61.0 % to 82.4 % of nominal at test initiation and from 24.4% to 64.5% of nominal at test termination. The analytical results are presented in Table 146.

Table 146: Analytical results

Nominal concentration (mg/L)	**Measured concentration (mg/L) 1 h**	**Recovery (%)**	**Measured concentration (mg/L) 96 h**	**Recovery (%)**
control	0.015	-	n.d.	-
solvent control	0.00131	-	n.d.	-
0.00464	0.00283	61.0	0.00113	24.4
0.00681	0.00488	71.7	0.00214	31.4
0.01	0.00739	73.9	0.00354	35.4
0.0147	0.00960	65.3	0.00452	30.7
0.0215	0.01501	69.8	0.00686	31.9
0.0316	0.02605	82.4	0.01312	41.5
0.0464	0.03712	80.0	0.02993	64.5

n.d. = not detectable

Biological results: BAS 500 F caused mortality to the bluegill at nominal concentrations ≥ 0.0316 mg as/L. Behavioural symptoms such as tumbling were monitored in the highest concentration tested (0.0464 mg as/L) before the fish died (see Table 147).

Table 147: Acute toxicity (96 h) of pyraclostrobin to bluegill (*Lepomis macrochirus*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.00464**	**0.00681**	**0.0100**	**0.0147**	**0.0215**	**0.0316**	**0.0464**
Concentration [mg a.s./L] (mean measured)	--	--	0.00113	0.00214	0.00354	0.00452	0.00686	0.01312	0.0299
Mortality [%] (96 h)	0	0	0	0	0	0	0	0	100
Symptoms (after 4 h) *	none	none	none	none	none	none	none	none	T(1)
Endpoints [mg pyraclostrobin/L] (mean measured)									
LC_{50} (96 h)	> 0.0196 < 0.0335								
NOEC (96 h survival)	0.00686								

* T = tumbling, number in bracket = number of affected fish

Validity criteria according to OECD TG 203 (1992)	**Obtained in this study**

The mortality in the control(s) should not exceed 10% (or one if less than ten are used) at the end of the test	No fish died until test end
Constant conditions should be maintained as far as possible throughout the test and if necessary, semi-static or flow-through procedures should be used.	Remained constant
The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.	< 60% (4.0 – 10.3 mg/L)
There must be evidence, that the concentration of the substance being tested has been satisfactorily maintained, and preferably it should be at least 80% of the nominal concentration throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.	61.0 - 82.4% at test initiation and 25.4 - 64.5% at test end; therefore, results are based on mean measured concentrations

Failure in terms of meeting one validity criterion is marked in **bold.**

Conclusions

In a static acute toxicity study with the bluegill the LC_{50} (96 h) of pyraclostrobin was > 0.0196 and < 0.0335 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.0109 mg a.s./L (mean measured).

Three out of the four validity criteria were met. Oxygen saturation decreased to < 60% in all treatments after 96 hours. However, this was observed in all treatments, oxygen concentrations remained constant between the individual treatments and were < 60% till 72 hours. It is noted that the fish size was greater than recommended in the OECD TG 203 (1992); *i.e.* average total length was 6.31 cm (5.5 – 7.0 cm; recommended: 2.0 ± 1.0 cm). The loading recommendation of the guideline was still followed. This point has to be considered in the frame of the risk assessment as the fish size presents a reflection of the age and the age of the fish can have an impact on sensitivity.

The validity criteria according to current OECD 203 version (2019) do not really deviate from the former version of 1992 thus all criteria with exception of the oxygen saturation were met. Regarding the criterion oxygen saturation the decrease at the end of the study and in all treatments and control can be considered to have no impact on the outcome of the study. Especially as a second acute study with *Lepomis macrochirus*, resulting in nearly the same endpoints and fulfilling all validity criteria, is available.

The study can still be considered to be valid. However, due to the decreased oxygen saturation and the considerable deviation in fish size, the study is considered as reliable with restrictions. It is relevant for classification purposes.

11.5.1.3 Acute toxicity to fish – *Cyprinus carpio*

Author:	Anonymous
Title:	Acute toxicity study on the common carp (*Cyprinus carpio* L.) in a static system (96 hours)
Date:	09.10.1998
Doc ID:	11F0494/965178; BASF DocID 1998/11580
Guidelines:	EPA 72-1, EEC 92/69, OECD 203
GLP:	Yes
Validity:	Acceptable
Previous evaluation:	In initial monograph

Material and Methods

Test item:	BAS 500 F (96/494-1, batch no. LJ 27882/191/C); purity: 97.1 %
Test species:	Common carp (*Cyprinus carpio* L.) scaly variety, mean body length 7.23 (4.5 – 7.7) cm; mean body weight 6.42 (4.6 – 7.2) g.
Test design:	Static system (96 hours); 10 fish per aquarium (loading about 0.6 g fish/L) and per concentration. Six test item concentrations, a solvent control and a negative (water) control; 1 replicates per treatment and control; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and after 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Control, solvent control, 0.01, 0.0147, 0.0215, 0.0316, 0.0464 and 0.0681 mg as/L (nominal), stock solution in acetone.
Test conditions:	Glass aquaria (80 x 35 x 46 cm), test volume: 100 L in a temperature-controlled room; temperature: 22 - 24 °C; pH 7.8 – 8.3; lowest dissolved oxygen concentration at test end 3.8 mg/L; photoperiod: 16 h light : 8 h dark; no feeding.
Analytics:	HPLC
Statistics:	No details presented in study report.

Results and Discussion

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration one hour after test start and, except for the highest concentration at the end of the test. The analyzed contents of pyraclostrobin ranged from 57.6% to 66.8% of nominal at test initiation and from 9.7% to 44.6% of nominal at test termination. The analytical results are presented in Table 148.

Table 148: Analytical results

Nominal concentration (mg/L)	**Measured concentration (mg/L) 1 h**	**Recovery (%)**	**Measured concentration (mg/L) 96 h**	**Recovery (%)**
control	0.000052	-	0.000184	-
solvent control	n.d.		n.d.	
0.01	0.00668	66.8	0.00176	17.6
0.0147	0.00847	57.6	0.00142	9.7
0.0215	0.01344	62.5	0.00301	14.0
0.0316	0.02058	65.1	0.00367	11.6
0.0464	0.03087	66.5	0.02068	44.6
0.0681	0.04427	65.0	*	-

n.d. = not detectable

* not measured as all fish were dead after 48 hours

Biological results: BAS 500 F caused mortality to the common carp at nominal concentrations ≥ 0.0464 mg as/L. Behavioural symptoms such as apathy, tumbling and narcosis-like state were monitored at the test concentrations 0.0464 mg as/L and 0.0681 mg as/L before the fish died.

Table 149: Acute toxicity (96 h) of pyraclostrobin to common carp (*Cyprinus carpio*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.01**	**0.0147**	**0.0215**	**0.0316**	**0.0464**	**0.0681**
Concentration [mg a.s./L] (mean measured)	--	--	0.00420	0.00490	0.00820	0.01210	0.02580	-
Mortality [%] (96 h)	0	0	0	0	0	0	100	100 (after 48 h)
Symptoms (after 48 h) *	none	none	none	none	none	none	N(2), T(1)	-
Endpoints [mg pyraclostrobin/L] (mean measured)								
LC_{50} (96 h)	> 0.0121 < 0.0258							
NOEC (96 h survival, sublethal effects)	0.0121							

* N = narcotic like stage, T = tumbling, number in brecket = number of affected fish

Validity criteria according to OECD TG 203 (1992)	**Obtained in this study**
The mortality in the control(s) should not exceed 10% (or one if less than ten are used) at the end of the test	No fish died until test end
Constant conditions should be maintained as far as possible throughout the test and if necessary, semi-static or flow-through procedures should be used.	Remained constant
The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.	< 60% (3.8 – 9.6 mg/L)
There must be evidence, that the concentration of the substance being tested has been satisfactorily maintained, and preferably it should be at least 80% of the nominal concentration throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.	57.6 - 66.8% at test initiation and 9.7 - 44.6% at test end; therefore, results are based on mean measured concentrations

Failure in terms of meeting one validity criterion is marked in **bold.**

Conclusions

In a static acute toxicity study with the common carp the LC_{50} (96 h) of pyraclostrobin was > 0.0121 and < 0.0258 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.0121 mg a.s./L (mean measured).

Three out of the four validity criteria were met. Oxygen saturation decreased to < 60 % in some treatments between 24 and 96 hours. However, the deviations are not expected to have had an influence on the overall outcome of the study. Carp can tolerate lower oxygen concentrations and the treatments with the lowest oxygen measurements showed 100 % survival. It is noted that the fish size was greater than recommended in the OECD TG 203 (1992); *i.e.* average total length was 7.2 cm (4.5 – 7.7 cm; recommended: 3.0 ± 1.0 cm). The loading recommendation of the guideline was still followed. This point has to be considered in the frame of the risk assessment as the fish size presents a reflection of the age and the age of the fish can have an impact on sensitivity.

The validity criteria according to current OECD 203 version (2019) do not really deviate from the former version of 1992 thus all criteria with exception of the oxygen saturation were met. Regarding the criterion oxygen saturation the observed decrease can be considered to have no impact on the outcome of the study in this case.

The study can still be considered as valid. However, due to the decreased oxygen saturation and the considerable deviation in fish size, the study is considered as reliable with restrictions. It is relevant for classification purposes.

11.5.1.4 Acute toxicity to fish – *Oncorhynchus mykiss*

Author:	Anonymous
Title:	Flow-through acute toxicity of BAS 500F to the rainbow trout, *Oncorhynchus mykiss*
Date:	16.02.2000
Doc ID:	1946-BA; BASF Reg. Doc. Number 2000\5034, BASF Study Number 63928
Guidelines:	EPA 72-1(c),EPA 850.1075
GLP:	Yes
Validity:	Acceptable (performed according to US EPA guideline but OECD 203 criteria were met)
Previous evaluation:	Submitted for the purpose of renewal

Material and Methods

Test item:	Pyraclostrobin (BAS 500 F; Reg. no.: 304 428), batch no. 27882/191/C, purity: 97.09%
Test species:	Rainbow trout (*Oncorhynchus mykiss*), juveniles; mean body length of control fish: 39.2 mm; mean wet weight of control fish: 0.55 g; supplied by Thomas Fish Company, Anderson, California, USA.
Test design:	Flow through system (96 h); 5 test item concentrations plus a dilution water control and a solvent control, 2 replicates per treatment; 10 fish per aquarium (loading 0.37 g fish/L); assessment of mortality and sublethal effects directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.0013, 0.0022, 0.0036, 0.0060 and 0.010 mg pyraclostrobin/L (nominal), corresponding to mean measured concentrations of 0.0014, 0.0022, 0.0036, 0.0053 and 0.0090 mg a.s./L.
Test conditions:	20 L glass aquaria, test volume: 15 L; dilution water: deionized, carbon filtered and aerated water; flow rate: 6.1 volume additions per 24 hours on average in each test vessel; hardness: 40 mg $CaCO_3$/L; temperature: 11.1 - 13.0°C; pH 7.3 - 7.9; oxygen content: 10.4 mg/L - 10.6 mg/L; conductivity: 130 - 140 µmhos/cm; photoperiod 16 h light : 8 h dark; light intensity: approx. 52 foot candles; no aeration; no feeding.
Analytics:	HPLC-method with UV detection
Statistics:	Descriptive statistics; binomial/ nonlinear interpolation for calculation of LC_{50}.

Results and Discussion

Analytical measurements: Analytical verification of pyraclostrobin concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of pyraclostrobin ranged from 86 to 115% of nominal at test initiation and from 88 to 111% of nominal at test termination. However the following biological results are based on mean measured concentrations. The results of the chemical analysis are shown in Table 150 (extracted from study report).

Table 150: Analytical results

Nominal Concentration of BAS 500 F (µg/L)	Replicate	Measured Concentration of BAS 500 F (µg/L) 0 Hour	96 Hour	Mean	Percent of Nominal
Test Media					
0 (control)	1	ND[1,2]	ND		
	2	ND	ND	ND	--
0 (solvent control)	1	ND	ND		
	2	ND	ND	ND	--
1.3	1	1.35, 1.34[3]	1.18		
	2	1.70, 1.66[3]	1.40	1.44	111
2.2	1	1.87	2.01		
	2	2.61	2.33	2.21	100
3.6	1	3.49	3.32		
	2	4.05	3.53	3.60	100
6.0	1	4.93	5.18		
	2	5.41	5.47	5.25	88
10	1	8.77	9.35		
	2	9.02	8.74	8.97	90
Blank					
0		ND	ND	ND	--
Matrix Spike Sample[4]					
3.6		3.93	3.87		
		4.05	3.65	3.88	108
Laboratory Control Sample[4]					
3.6		3.40	3.55	3.48	97
Secondary Stock Solution					
10		8.85	9.13	8.99	90

[1] ND = none detected at or above the limit of quantitation of 0.50 µg/L.
[2] Test substance was detected above the limit of detection (LOD) but below the limit of quantification (LOQ). Measured concentrations in the replicate 1 control vessel at 0 hour (0.360 and 0.380 µg/L) and at 96 hours (0.170 and 0.160 µg/L) are approximations as the measured concentrations are less than the LOQ.
[3] Sample was reanalyzed due to high initial value.
[4] Matrix spike samples and laboratory control samples were prepared with the test substance used to formulate the test concentrations (purity = 97.09%).

Biological results: After 96 hours of exposure, no mortality was observed in the dilution water control, the solvent control and at test item concentrations of up to and including 0.0036 mg a.s./L, whereas 20 % mortality was observed at 0.0053 mg a.s./L. At the highest tested concentration, all fish were dead after 96 hours of exposure. Sub-lethal effects (i.e. change in coloration) were found at 0.0053 mg a.s./L after 96 hours. The results are summarized in Table 151: .

Table 151: Acute toxicity (96 h) of pyraclostrobin to rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0013**	**0.0022**	**0.0036**	**0.0060**	**0.010**
Concentration [mg a.s./L] (mean measured)	--	--	0.0014	0.0022	0.0036	0.0053	0.0090
Mortality [%] (96 h)	0	0	0	0	0	20	100
Symptoms (after 96 h) *	none	none	none	none	none	C	n.d.
Endpoints [mg pyraclostrobin/L] (mean measured)							
LC_{50} (96 h)	0.0062 (95% confidence limits: 0.0053 - 0.0090)						

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0013**	**0.0022**	**0.0036**	**0.0060**	**0.010**
Concentration [mg a.s./L] (mean measured)	--	--	0.0014	0.0022	0.0036	0.0053	0.0090
NOEC (96 h)	0.0036						

n.d. = not determined; all fish dead
* Symptoms after 96 h: C = change in coloration.

Validity criteria according to OECD TG 203 (1992)	**Obtained in this study**
The mortality in the control(s) should not exceed 10% (or one if less than ten are used) at the end of the test	No fish died until test end
Constant conditions should be maintained as far as possible throughout the test and if necessary, semi-static or flow-through procedures should be used.	Remained constant
The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.	> 60% (10.4 – 10.6 mg/L)
There must be evidence, that the concentration of the substance being tested has been satisfactorily maintained, and preferably it should be at least 80% of the nominal concentration throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.	86 to 115% of nominal throughout the test

Conclusions

In a flow-through acute toxicity study with rainbow trout the LC_{50} (96 h) of pyraclostrobin was 0.0062 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.0036 mg a.s./L.

It is noted that the fish size was somewhat smaller than recommended in the OECD TG 203 (1992); *i.e.* average total length was 3.9 cm (recommended: 5.0 ± 1.0 cm). The loading recommendation of the guideline was still followed. In the frame of the risk assessment it has to be considered that there were other fish acute studies with a clearly higher deviation of the size. As the fish size presents a reflection of the age and the age of the fish can have an impact on sensitivity this point should be taken into account for risk assessment. Regarding the fish size, the deviation from recommended size in this certain case is rather small. The validity criteria of the new version of OECD 203 from 2019 are covered and fulfilled.

The study can still be considered as valid and reliable. It is relevant for classification purposes.

Acute toxicity to fish – *Cyprinodon variegatus*

Author:	Anonymous
Title:	Flow-through acute toxicity of BAS 500F to the sheepshead minnow, *Cyprinodon variegatus*
Date:	15.02.2000
Doc ID:	1317-BA; BASF Reg. Doc. Number 2000\5032, BASF Study Number 97112
Guidelines:	EPA 72-1(c), EPA 850.1075
GLP:	Yes
Validity:	Acceptable (according to US EPA guideline, but also OECD 203 criteria were met)
Previous evaluation:	Submitted for the purpose of renewal

Material and Methods

Test item:	Pyraclostrobin (BAS 500 F; Reg. no.: 304 428), batch no. 27882/191/C, purity: 97.09%

Test species:	Sheepshead minnow (*Cyprinodon variegatus*), juveniles; mean body length of control fish: 21.0 mm; mean wet weight of control fish: 0.16 g; supplied by "Aquatic BioSystems", Fort Collins, Colorado, USA.
Test design:	Flow through system (96 h); 5 test item concentrations plus a dilution water control and a solvent control; 2 replicates per treatment; 10 fish per aquarium (loading 0.11 g fish/L); assessment of mortality and sublethal effects directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.013, 0.022, 0.036, 0.060 and 0.10 mg pyraclostrobin/L (nominal), corresponding to mean measured concentrations of 0.0108, 0.0188, 0.0302, 0.0535 and 0.0879 mg a.s./L.
Test conditions:	20 L glass aquaria, test volume: 15 L; dilution water: filtered natural seawater mixed with deionized water, salinity: 15 - 16 ‰; flow rate: 5.7 volume additions per 24 hours on average per test vessel; temperature: 21.8°C- 22.8°C; pH 7.8 - 7.9; oxygen content: 7.4 mg/L - 8.1 mg/L; photoperiod 16 h light : 8 h dark; light intensity: approx. 53 foot candles; no aeration; no feeding.
Analytics:	HPLC-method with UV detection
Statistics:	Descriptive statistics; binomial/ nonlinear interpolation for calculation of LC_{50}.

Results and Discussion

Analytical measurements: Analytical verification of pyraclostrobin concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of pyraclostrobin ranged from 82 to 89 % of nominal at test initiation and from 83 to 89% of nominal at test termination. However the biological results are based on mean measured concentrations. The results of the chemical analysis are shown in Table 152 (extracted from study report):

Table 152: Analytical results

Nominal Concentration of BAS 500 F (µg/L)	Replicate	Measured Concentration of BAS 500 F (µg/L)[1] 0 Hour	96 Hour	Mean	Percent of Nominal
Test Media					
0 (control)	1	ND[2]	ND		
	2	ND	ND	ND	--
0 (solvent control)	1	ND	ND		
	2	ND	ND	ND	--
13	1	10.6	10.9		
	2	10.8	11.0	10.8	83
22	1	19.3	19.1		
	2	19.2	17.7	18.8	85
36	1	30.5	30.3		
	2	29.2	30.7	30.2	84
60	1	52.9	53.8		
	2	53.6	53.6	53.5	89
100	1	86.3	88.0		
	2	87.3	89.8	87.9	88
Blank					
0		ND	ND	ND	--
Matrix Spike Sample[3]					
36		34.6	35.7		
		35.1	36.2	35.4	98
Laboratory Control Sample[3]					
36		35.8	35.6	35.7	99
Secondary Stock Solution					
100		87.7	83.3	85.5	86

[1] The analysis of pretest samples collected one day prior to the start of the test from test vessels with nominal concentrations of 0 (control), 13, 36, and 100 µg/L resulted in measured values of <0.50, 10.1, 30.5, and 85.9 µg/L, respectively.
[2] ND = none detected at or above the limit of quantitation of 0.50 µg/L.
[3] Matrix spike samples and laboratory control samples were prepared with the test substance used to formulate the test concentrations (purity = 97.09%).

Biological results: After 96 hours of exposure, no mortality was observed in the dilution water control, the solvent control and at test item concentrations of up to and including 0.0535 mg a.s./L, whereas 75% mortality was observed at the highest tested concentration of 0.0879 mg a.s./L. No sub-lethal effects were found in the control groups and in all test item treatments after 96 hours. The results are summarized in Table 153.

Table 153: Acute toxicity (96 h) of pyraclostrobin to sheepshead minnow (*Cyprinodon variegatus*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.013**	**0.022**	**0.036**	**0.060**	**0.10**
Concentration [mg a.s./L] (mean measured)	Control	Solvent control	0.0108	0.0188	0.0302	0.0535	0.0879
Mortality [%] (96 h)	0	0	0	0	0	0	75
Symptoms (after 96 h)	none	none	none	none	none	none	none
Endpoints [mg pyraclostrobin/L] (mean measured)							
LC_{50} (96 h)	0.0769 (95% confidence limits: 0.0535 - 0.0879)						

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.013**	**0.022**	**0.036**	**0.060**	**0.10**
Concentration [mg a.s./L] (mean measured)	Control	Solvent control	0.0108	0.0188	0.0302	0.0535	0.0879
NOEC (96 h)	0.0535						

Validity criteria according to OECD TG 203 (1992)	**Obtained in this study**
The mortality in the control(s) should not exceed 10% (or one if less than ten are used) at the end of the test	No fish died until test end
Constant conditions should be maintained as far as possible throughout the test and if necessary, semi-static or flow-through procedures should be used.	Remained constant
The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.	> 60% (7.4 – 8.1 mg/L)
There must be evidence, that the concentration of the substance being tested has been satisfactorily maintained, and preferably it should be at least 80% of the nominal concentration throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.	82 to 89% of nominal throughout the test

Conclusions

In a flow-through acute toxicity study with sheepshead minnow the LC50 (96 h) of pyraclostrobin was 0.0769 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.0535 mg a.s./L. All validity criteria were met. Criteria in the current OECD 203 version (2019) do not really deviate to former version (1992) and are all met.

The study is valid and reliable. It is relevant for classification purposes.

11.5.1.5 Acute toxicity to fish – *Lepomis macrochirus*

Author:	Anonymous
Title:	Flow-through acute toxicity of BAS 500F to the bluegill sunfish, *Lepomis macrochirus*
Date:	17.02.2000
Doc ID:	1947-BA; BASF Reg. Doc. Number 2000\5033, BASF Study Number 63930
Guidelines:	EPA 72-1(c), EPA 850.1075
GLP:	Yes
Validity:	Acceptable (performed according to US EPA guideline but OECD 203 criteria were met)
Previous evaluation:	Submitted for the purpose of renewal

Material and Methods

Test item:	Pyraclostrobin (BAS 500 F; Reg. no.: 304 428), batch no. 27882/191/C, purity: 97.09 %
Test species:	Bluegill sunfish (*Lepomis macrochirus*), juveniles; mean body length of control fish: 23.8 mm; mean wet weight of control fish: 0.11 g; supplied by "Osage Catfisheries", Osage Beach, Missouri, USA.
Test design:	Flow through system (96 h); 5 test item concentrations plus a dilution water control and a solvent control, 2 replicates per treatment; 10 fish per aquarium (loading 0.073 g fish/L);

	assessment of mortality and sublethal effects directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.0065, 0.011, 0.018, 0.030 and 0.050 mg pyraclostrobin/L (nominal), corresponding to mean measured concentrations of 0.0061, 0.0095, 0.0159, 0.0269 and 0.0451 mg a.s./L.
Test conditions:	20 L glass aquaria, test volume: 15 L; dilution water: deionized, carbon filtered and aerated water, flow rate: 6.3 volume additions per 24 hours on average in each test vessel; hardness: 48 mg $CaCO_3$/L; temperature: 21.2°C - 22.5°C; pH 7.3 - 7.9; oxygen content: 8.7 mg/L - 9.4 mg/L; conductivity: 130 - 160 µmhos/cm; photoperiod: 16 h light : 8 h dark; light intensity: approx. 52 foot candles; no aeration; no feeding.
Analytics:	HPLC-method with UV detection
Statistics:	Descriptive statistics; binomial/ nonlinear interpolation for calculation of LC_{50}.

Results and Discussion

Analytical measurements: Analytical verification of pyraclostrobin concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of pyraclostrobin ranged from 85 to 93% of nominal at test initiation and from 85% to 94% of nominal at test termination. However the following biological results are based on mean measured concentrations. The results of the chemical analysis are shown in Table 154 (extracted from study report):

Table 154: Analytical results

Nominal Concentration of BAS 500 F (µg/L)	Replicate	Measured Concentration of BAS 500 F (µg/L)[1] 0 Hour	96 Hour	Mean	Percent of Nominal
Test Media					
0 (control)	1	ND[2]	ND		
	2	ND	ND	ND	--
0 (solvent control)	1	ND[3]	ND		
	2	ND[3]	ND	ND	--
6.5	1	6.05	6.05		
	2	6.09	6.12	6.08	94
11	1	9.27	10.0		
	2	9.48	9.32	9.52	87
18	1	15.4	16.4		
	2	15.2	16.5	15.9	88
30	1	26.0	27.9		
	2	25.8	28.0	26.9	90
50	1	44.3	46.0		
	2	43.7	46.5	45.1	90
Blank					
0		ND	ND	ND	--
Matrix Spike Sample[4]					
18		17.8	18.6		
		18.3	18.3	18.3	102
Laboratory Control Sample[4]					
18		18.2	18.7	18.5	103
Secondary Stock Solution					
50		46.4	46.4	46.4	93

[1] The analysis of pretest samples collected one day prior to the start of the test from test vessels with nominal concentrations of 0 (control), 6.5, 18, and 50 µg/L resulted in measured values of <0.50, 6.37, 15.3, and 42.7 µg/L, respectively.

[2] ND = none detected at or above the limit of quantitation of 0.50 µg/L.

[3] Test substance was detected above the limit of detection (LOD) but below the limit of quantification (LOQ). Measured concentrations (0.269 and 0.425 µg/L) are approximations as their areas are less than the area of the lowest calibration standard.

[4] Matrix spike samples and laboratory control samples were prepared with the test substance used to formulate the test concentrations (purity = 97.09%).

Biological results: After 96 hours of exposure, no mortality was observed in the dilution water control, the solvent control and at the lowest test item concentration of 0.0061 mg a.s./L, whereas 15% mortality was observed at 0.0095 mg a.s./L. At the three highest test item concentrations, all fish were dead after 96 hours of exposure. After 96 hours of exposure, sub-lethal effects (i.e. lethargy and loss of equilibrium) were found at 0.0095 mg a.s./L. The results are summarized in Table 155: .

Table 155: Acute toxicity (96 h) of pyraclostrobin to bluegill sunfish (*Lepomis macrochirus*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0065**	**0.011**	**0.018**	**0.030**	**0.050**
Concentration [mg a.s./L] (mean measured)	--	--	0.0061	0.0095	0.0159	0.0269	0.0451
Mortality [%] (96 h)	0	0	0	15	100	100	100
Symptoms (after 96 h) *	none	none	none	L	n.d.	n.d.	n.d.

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0065**	**0.011**	**0.018**	**0.030**	**0.050**
Concentration [mg a.s./L] (mean measured)	--	--	0.0061	0.0095	0.0159	0.0269	0.0451
Endpoints [mg pyraclostrobin/L] (mean measured)							
LC_{50} (96 h)	0.0114 (95% confidence limits: 0.0095 - 0.0159)						
NOEC (96 h)	0.0061						

n.d. = not determined; all fish dead
* Symptoms after 96 h: L = lethargy and loss of equilibrium.

Validity criteria according to OECD TG 203 (1992)	**Obtained in this study**
The mortality in the control(s) should not exceed 10% (or one if less than ten are used) at the end of the test	No fish died until test end
Constant conditions should be maintained as far as possible throughout the test and if necessary, semi-static or flow-through procedures should be used.	Remained constant
The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.	> 60% (8.7 – 9.4 mg/L)
There must be evidence, that the concentration of the substance being tested has been satisfactorily maintained, and preferably it should be at least 80% of the nominal concentration throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.	85 to 94% of nominal throughout the test

Conclusions

In a flow-through acute toxicity study with bluegill sunfish the LC_{50} (96 h) of pyraclostrobin was 0.0114 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.0061 mg a.s./L.

All validity criteria were met. Criteria in the current OECD 203 version (2019) do not really deviate to former version (1992) and are all met.

The study is valid and reliable. It is relevant for classification purposes.

11.5.1.6 Acute toxicity to fish – *Pimephales promelas*

Author:	Anonymous
Title:	BAS 500 F (Pyraclostrobin) - Acute toxicity study in the fathead minnow (*Pimephales promelas*)
Date:	18.12.2014
Doc ID:	18F0494/96E001; BASF DocID 2014/1238538
Guidelines:	OECD 203, US EPA 850.1075, EPA 72-1
GLP:	Yes
Validity:	Acceptable
Previous evaluation:	Submitted for the purpose of renewal

Material and Methods

Test item:	Pyraclostrobin (BAS 500 F; Reg. No. 304428); batch no.: COD-001236; purity: 99.02 ± 1.0%.

Test species:	Fathead Minnow (*Pimephales promelas*), approx. 7 months old, mean body length 4.7 cm (4.2 – 5.1 cm), mean body weight 0.98 g (0.73 – 1.38 g); in-house culture
Test design:	Flow-through (96 hours); five test item concentrations plus a control in 2 replicate per treatment; 10 fish per replicate (loading about 0.18 g fish/L); assessment of survival and symptoms of toxicity after 1, 6, 24, 48, 72 and 96 hours.
Endpoints:	LC_{50} and NOEC based on mortality and sublethal effects.
Test concentrations:	Control, 0.0053, 0.0080, 0.012, 0.018 and 0.027 mg pyraclostrobin/L (nominal), corresponding to mean measured concentrations of 0.0044, 0.0071, 0.0101, 0.0163 and 0.0239 mg a.s./L.
Test conditions:	Stainless steel aquaria; test volume 9 L, dilution water: non-chlorinated, charcoal filtered drinking water mixed with deionized water; flow rate: 36.5 – 38.0 mL/min; temperature: 23.8 – 24.2°C; pH 8.0 - 8.1; oxygen content: 7.3 - 8.2 mg/L (>75% saturation); total hardness: 1.02 mmol/L; conductivity: 258 µS/cm (at 25°C); photoperiod: 16 hours light : 8 hours dark; light intensity: approx. 76 – 675 lux; no aeration; no feeding.
Analytics:	Analytical verification of the test item concentrations was performed using an HPLC-method with MS detection.
Statistics:	Descriptive statistics, probit analysis for determination of LC_x-values.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning of the test, after 48 hours and at test termination. The analytically detected concentrations were initially within a range of 87 to 95% of the nominal values, between 77 and 86% after 48 h and between 82 and 102% at the end of the test. The mean measured concentrations were between 83 and 91% of the nominal throughout the test. The biological results are based on mean measured concentrations. The results of the chemical analysis are shown in Table 156 (extracted from study report):

Table 156: Analytical results

The analytically determined concentrations of the test substance during exposure in the aquaria were:

Nominal Concentration	Sample Number	Sampling Time	Measured Concentration			Mean Measured Concentration	
[mg a.i./L]		(days)	[mg a.i./L]	% Nominal	max/min	[mg a.i./L]	% Nominal
0 (Control)	1	0	< LOQ	–	–	< LOQ	–
	2	0	< LOQ	–	–		
	21	2	< LOQ	–	–		
	22	2	< LOQ	–	–		
	41	4	< LOQ	–	–		
	42	4	< LOQ	–	–		
0.0053	3	0	0.00490	93%	1.2	0.0044	83%
	4	0	0.00460	87%			
	23	2	0.00407	77%			
	24	2	0.00406	77%			
	43	4	0.00447	84%			
	44	4	0.00439	83%			
0.008	5	0	0.00759	95%	1.2	0.0071	89%
	6	0	0.00754	94%			
	25	2	0.00680	85%			
	26	2	0.00652	81%			
	45	4	0.00720	90%			
	46	4	0.00711	89%			
0.012	7	0	0.0110	92%	1.2	0.0101	84%
	8	0	0.0109	91%			
	27	2	0.00955	80%			
	28	2	0.00919	77%			
	47	4	0.00998	83%			
	48	4	0.00982	82%			
0.018	9	0	0.0166	92%	1.3	0.0163	91%
	10	0	0.0167	93%			
	29	2	0.0148	82%			
	30	2	0.0144	80%			
	49	4	0.0184	102%			
	50	4	0.0170	95%			
0.027	11	0	0.0250	93%	1.1	0.0239	89%
	12	0	0.0242	90%			
	31	2	0.0231	86%			
	32	2	0.0228	84%			
	51	4	0.0242	90%			
	52	4	0.0243	90%			

Biological results: After 96 hours no mortality occurred in the control and at the test item concentrations of up to and including 0.0101 mg a.s./L, whereas 90 and 100% mortality occurred at the two highest concentrations of 0.0163 and 0.0239 mg a.s./L, respectively. No behavioral abnormalities and toxic signs were observed at any test item concentration after 96 hours of exposure. The results are summarized in Table 157.

Table 157: Acute toxicity (96 h) of pyraclostrobin to fathead minnow (*Pimephales promelas*)

Concentration [mg a.s./L] (nominal)	**Control**	**0.0053**	**0.008**	**0.012**	**0.018**	**0.027**
Concentration [mg a.s./L] (mean measured)	-	0.0044	0.0071	0.0101	0.0163	0.0239
Mortality [%] (96 h)	0	0	0	0	90	100
Symptoms (96 h) *	none	none	none	none	none	n.d.
Endpoints [mg pyraclostrobin/L] (mean measured)						
LC_{50} (96 h)	0.0146 (95% confidence limits: n.d.)					
NOEC (96 h)	0.0101					

* Symptoms after 96 h: n.d. = not determined; all fish dead

Validity criteria according to OECD TG 203 (1992)	**Obtained in this study**

The mortality in the control(s) should not exceed 10% (or one if less than ten are used) at the end of the test	No fish died until test end
Constant conditions should be maintained as far as possible throughout the test and if necessary, semi-static or flow-through procedures should be used.	Remained constant
The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test.	> 60% (7.3- 8.2 mg/L mg/L)
There must be evidence, that the concentration of the substance being tested has been satisfactorily maintained, and preferably it should be at least 80% of the nominal concentration throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentrations.	0 h: 87% - 95% 48 h: 77% - 86% 96 h: 82% and 102%; therefore, results are based on mean measured concentrations

Conclusions

In a 96-hours flow-through acute toxicity study with fathead minnow the LC_{50} (96 h) for pyraclostrobin was calculated to be 0.0146 mg a.s./L based on mean measured concentrations. The NOEC was determined to be 0.0101 mg a.s./L (mean measured).

It is noted that the fish size was exceeding the OECD TG 203 (1992) recommendations; *i.e.* average total length was 4.7 cm (4.2 – 5.1 cm; recommended: 2.0 ± 1.0 cm). The loading recommendations of the guideline were still followed. This point has to be considered in the frame of the risk assessment as the fish size presents a reflection of the age and the age of the fish can have an impact on sensitivity.

All validity criteria were met. Criteria in the current OECD 203 version (2019) do not really deviate to former version (1992) and are all met.

The study is valid. However, due to the considerable deviation in fish size, the study is considered as reliable with restrictions. It is relevant for classification purposes.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

11.5.2.1 Acute toxicity to invertebrates – *Daphnia magna*

Author:	Dohmen, G.P.
Title:	Effect of BAS 500 F on *Daphnia magna* STRAUS in a 48 hours acute toxicity test
Date:	07.06.1999
Doc ID:	35806; Reg. Doc. # BASF 1999/10444 (combined with Amendment 1999/10739)
Guidelines:	OECD 202, EEC 92/69
GLP:	Yes
Validity:	Acceptable
Previous evaluation:	In initial DAR (2001)

Material and Methods

Test item:	BAS 500 F (batch J. No. 27882/191/c), purity: 97.1 %
Test species:	Waterflea (*Daphnia magna* STRAUS), neonates with age at test initiation less than 24 hours.

Test design:	Static test (48 hours), six test concentrations plus control, 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.
Endpoints:	EC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Control, 0.005, 0.0076, 0.0115, 0.0174, 0.0264 and 0.04 mg as/L (nominal)
Test conditions:	Water temperature was measured to be 20.3 – 20.4 °C. Dissolved oxygen concentrations was 8.9 mg/L at the beginning (0 h) and 9.0 mg/L at the end (48 h) of the test. Measurements of pH ranged from 8.07 to 8.16 (0 h) and 8.04 to 8.08 (48 h). Light intensity of < 1500 lux with 16 hours light and 8 hours darkness.
Analytics:	RP-HPLC method CP 277
Statistics:	Descriptive statistics; probit analysis for calculation of the 48-h EC_{50}; determination of NOEC by visual interpretation of observation data.

Results and Discussion

Analytical measurements: Analytical verification of the test substance was carried out in each concentration at the beginning and the end of the test. The measured concentrations ranged from 95.6% to 105.4% of nominal at test initiation and from 89.0% to 107.6% of nominal at the end of the test. Hence the endpoints were based on nominal concentrations. The results of the chemical analysis are shown in Table 158:

Table 158: Analytical results

Nominal concentration (mg/L)	**Measured concentration (mg/L) 0 h**	**% of nominal**	**Measured concentration (mg/L) 48 h**	**% of nominal**
0.0050	0.00527	105.4	0.00495	99.1
0.0076	0.00780	102.6	0.00746	98.2
0.0115	0.01181	102.7	0.01089	94.7
0.0174	0.01790	102.9	0.01549	89.0
0.0264	0.02662	100.8	0.02840	107.6
0.0400	0.03826	95.6	0.03821	95.5
0	n.d.	-	-	-

Biological results: The dose-response curve for BAS 500 F is steep. No significant immobility (one Daphnia after 48 h) was observed at 0.0115 mg as/L. At 0.0174 mg as/L more than half of the daphnids were immobile after 48 hours and all daphnids were immobile at 0.0264 mg as/L (Table 159). No other effects were observed.

Table 159: Effect of BAS 500 F on *Daphnia magna*

Concentration [mg a.s./L] (nominal)	**control**	**0.005**	**0.0076**	**0.0115**	**0.0174**	**0.0264**	**0.0400**
Immobility (48 h) [%]	0	0	0	5	70	100	100
Endpoints [mg pyraclostrobin/L] (nominal)							
EC_{50} (48 h)	0.0157 (95% confidence limits: 0.0144 – 0.0172)						
NOEC (48 h)	0.0115						

Validity criteria according to OECD TG 202 (2004)	**Obtained in this study**

In the control, including the control containing the solubilising agent, not more than 10% of the daphnids should have been immobilized. (Not more than 10% of the control daphnids should show immobilization or other signs of disease or stress, for example, discoloration or unusual behaviour such as trapping at surface of water.)	0%
The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels.	8.9 – 9.0 mg/L

Conclusions

In a 48-hour static acute limit test with *Daphnia magna* the EC_{50} of pyraclostrobin was determined to be 0.0157 mg a.s./L based on nominal concentrations. The NOEC was 0.0115 mg a.s./L (nominal).

The study is considered as valid and reliable. It is relevant for classification purposes.

11.5.2.2 Acute toxicity to invertebrates – *Americamysis bahia*

Author:	Boeri, R.L. et al.
Title:	Flow-Through Acute Toxicity of BAS 500 F to the Mysid, *Americanysis bahia*
Date:	15.02.2000
Doc ID:	1318-BA; BASF Reg. Doc. Number 2000/5031, study number 97114
Guidelines:	EPA 850.1035, EPA 72-3(b)
GLP:	Yes
Validity:	Acceptable
Previous evaluation:	Submitted for the purpose of renewal

Material and Methods

Test item:	Pyraclostrobin (BAS 500 F; Reg. no.: 304 428), batch no. 27882/191/C; purity: 97.09%.
Test species:	Saltwater mysid (*Americamysis bahia*), juveniles, age: less than 24 hours old; average wet weight of control mysids: 0.88 mg; source: in-house cultures.
Test design:	Flow-through system (96 hours); 5 test item concentrations plus a control and a solvent control, 2 replicates per treatment; 10 mysids per replicate (loading 0.00059 g mysid/L); assessment of mortality and symptoms of toxicity directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50} (96 and 48 h), NOEC, mortality and sub-lethal effects.
Test concentrations:	Control (dilution water), solvent control (0.1 mL dimethylformamide/L) and 0.0010, 0.0018, 0.0029, 0.0048 and 0.0080 mg pyraclostrobin/L (nominal), corresponding to mean measured concentrations of 0.00079, 0.0014, 0.0021, 0.0036 and 0.0060 mg pyraclostrobin/L.
Test conditions:	Glass aquaria (20 L), test volume 15 L; test chambers: glass cylinders (8 cm in height and 8 cm in diameter) with mesh screen attached to the bottom; dilution water: filtered, sterilized and aerated natural seawater mixed with deionized water; flow rate: 6.5 volume additions per 24 hours on average; salinity: 16 - 17‰; temperature: 21.2°C - 22.9°C; pH 7.9 - 8.1; oxygen content: 7.9 - 8.3 mg/L; photoperiod 16 h light : 8 h dark with a 15 minute transition period between dark and light; light intensity: 55 foot-candles; feeding: juvenile mysids were fed daily with brine shrimps (*Artemia salina*); no aeration.
Analytics:	HPLC-method with UV-detection
Statistics:	Descriptive statistics; binomial/nonlinear interpolation method for calculation of the LC_{50}.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The analytically determined concentrations of pyraclostrobin ranged from 66.8 to 78.0% of nominal concentrations at test initiation and from 66.8 to 83.2% of nominal at test termination. Thus the biological results are based on mean measured concentrations. The results of the chemical analysis are shown in Table 160:

Table 160: Analytical results

Nominal concentration (mg/L)	**Measured concentration (mg/L)**				**Percent of nominal**
	Replicate	**0 hour**	**96 hour**	**Mean**	
Control	1 2	n.d. n.d.	n.d. n.d.	n.d.	--
Solvent control	1 2	n.d. n.d.	n.d. n.d.	n.d.	--
0.0010	1 2	0.000758 0.000766	0.000800 0.000832	0.000789	79
0.0018	1 2	0.000129 0.000138	0.000137 0.000132, 0.00014 [1]	0.00135	75
0.0029	1 2	0.00210 0.00206	0.00219 0.00214	0.00212	73
0.0048	1 2	0.00361 0.00369	0.00349 0.00378	0.00364	76
0.0080	1 2	0.00541, 0.00621, 0.00596 [1] 0.00534, 0.00584, 0.00624 [1]	0.00534,0.00663,0.00664 [1] 0.00609	0.00597	75

n.d. = not detectable

[1] Sample was reanalyzed due to low initial result

Biological results: After 48 hours of exposure no mortality and no other toxic effects were observed in the control, the solvent control and in concentrations of up to and including 0.0036 mg/L, whereas 20% mortality was observed at the highest test item concentration of 0.0060 mg a.s./L. After 48 hours, no sub-lethal effects were found. The results are summarized in Table 161.

Table 161: Acute toxicity of pyraclostrobin to saltwater mysids (*Americamysis bahia*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0010**	**0.0018**	**0.0029**	**0.0048**	**0.0080**
Concentration [mg a.s./L] (mean measured)	--	--	0.00079	0.0014	0.0021	0.0036	0.0060
Mortality [%] (48 h)	0	0	0	0	0	0	20
Symptoms after 48 h	none	none	none	none	none	none	none
Mortality [%] (96 h)	0	0	0	0	0	25	100
Symptoms after 96 h	none	none	none	none	none	-	-
Endpoints [mg pyraclostrobin/L] (mean measured)							
LC_{50} (48 h)	> 0.00597						
LC_{50} (96 h)	0.00416 (95% confidence limits: 0.00364 – 0.00597)						
NOEC	0.00212						

Recommendations according to US EPA OPPTS 850.1035 (1996) *	**Obtained in this study**
The test is unacceptable if more than 10 percent of the control organisms die or exhibit abnormal behavior during the 96–h test period.	0%
Among replicate test chambers of a treatment concentration, the measured concentration of the test substance should not vary more than 20%.	< 20%
Dissolved oxygen concentration: between 60 - 105% of the air saturation value	Between 60 - 105% (7.9 – 8.3 mg/L)

* As no concrete validity criteria are defined in the EPA guideline 850.1035 (1996), "recommendations" of the guideline have been considered for the validity check congruently with the OECD TG 202 for acute testing of *Daphnia* sp. (crustacean).

Conclusions

In a flow-through acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (48 h) for pyraclostrobin was determined to be > 0.00597 mg a.s./L. Since the regular test duration according to US EPA 850.1035 is 96 hours, also the 96-hour endpoint of the study has to be considered: LC_{50} (96 h) = 0.00416 mg a.s./L (mean measured) (95% confidence limits: 0.00364 – 0.00597), NOEC (96 h) = 0.00212 mg a.s./L (mean measured.

The study is considered valid and reliable. It is relevant for classification purposes.

11.5.2.3 Acute toxicity to invertebrates – *Crassostrea virginica*

Author:	Boeri, R.L. et al.
Title:	Flow-through mollusc shell deposition test with BAS 500 F
Date:	23.02.2000
Doc ID:	1319-BA; BASF Reg. Doc. Number 2000/5042, study number 97113
Guidelines:	EPA 850.1025, EPA 72-3(c)
GLP:	Yes
Validity:	Acceptable
Previous evaluation:	Submitted for the purpose of renewal

Material and Methods

Test item:	Pyraclostrobin (BAS 500 F; Reg. no.: 304 428), batch no. 27882/191/C; purity: 97.09%.
Test species:	Eastern oyster (*Crassostrea virginica*), juveniles, height: 30 - 50 mm; source: "P. Cummins Oyster Company", Baltimore, Maryland, USA.
Test design:	Flow-through system (96 hours); 5 test item concentrations plus a control and a solvent control, 2 replicates for each test item concentration and the controls with 10 oysters per replicate (20 animals per treatment); initially and daily assessment of mortality and symptoms of toxicity; measurements of shell deposition 96 hours after start of exposure.
Endpoints:	EC_{50} and NOEC for shell growth inhibition, mortality and symptoms of toxicity.
Test concentrations:	Control (dilution water: unfiltered seawater), solvent control (0.1 mL dimethylformamide/L); 0.0033, 0.0055, 0.0090, 0.015 and 0.025 mg pyraclostrobin/L

	(nominal), corresponding to mean measured concentrations of 0.0027, 0.0041, 0.0065, 0.0128 and 0.0215 mg a.s./L.
Test conditions:	20 L glass aquaria, test volume 15 L, natural unfiltered seawater, flow rate: average of 8.7 volume additions per 24 hours in each test vessel, 0.54 L per oyster per hour; salinity: 34‰; temperature: 20.2°C - 22.0°C; pH 7.8 - 8.1; oxygen content: 5.5 mg/L - 8.5 mg/L; photoperiod 16 h light : 8 h dark with a 15 minute transition period between dark and light; light intensity: 50 foot candles; no aeration; live marine phytoplankton as supplement to existing food in unfiltered seawater used as dilution water.
Analytics:	HPLC-method with UV-detection
Statistics:	Descriptive statistics; t-test (a = 0.05) for comparison of shell deposition data in the control groups; standard statistical techniques for calculation of EC_{50}, ANOVA followed by Bonferroni's test for shell deposition data of the test item treatments.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at test initiation and at test termination. Mean measured concentrations for pyraclostrobin ranged from 73 to 89% of nominal concentrations at test initiation and from 72 to 85% of nominal at test termination. Thus the biological results are based on mean measured concentrations. The results of the chemical analysis are shown in Table 162.

Table 162: Analytical results

Nominal concentration (mg/L)	**Measured concentration (mg/L)**				**Percent of nominal**
	Replicate	**0 hour**	**96 hour**	**Mean**	
Control	1 2	n.d. [1] n.d.	n.d. n.d.	n.d.	--
Solvent control	1 2	n.d. [1] n.d.	n.d. [1] n.d.	n.d.	--
0.0033	1 2	0.00267 0.00252	0.00268 0.00272	0.00265	80
0.0055	1 2	0.00392 0.00412	0.00403 0.00417	0.00406	74
0.0090	1 2	0.00649 0.00665	0.00648 0.00639	0.00650	72
0.0150	1 2	0.01310 0.01360	0.01190 0.01250	0.01280	85
0.0250	1 2	0.02210 0.02160	0.02130 0.02100	0.02150	86

n.d. = not detectable

[1] > LOD but < LOQ (= 0.0005 mg/L)

Biological results: After 96 hours of exposure, survival of oysters was 95 and 100% in the control and the solvent control, respectively. No mortality occurred at test item concentrations of up to and including 0.0065 mg pyraclostrobin/L, whereas 5 and 10% mortality was observed at 0.0128 mg a.s./L and 0.0215 mg a.s./L, respectively. No sublethal effects were noted during the exposure period in the controls and the test item treatments. Control and solvent control oysters deposited an average of 2.3 and 2.5 mm of new shell during the test, respectively. No statistically significant difference in shell deposition was observed between the control groups (t-test, $\alpha = 0.05$). Subsequent statistical analyses were performed by comparing the pooled

control and solvent control data to the treatment data. Statistically significant inhibition of shell growth compared to the pooled control / solvent control was observed at the three highest tested concentrations (Bonferroni's test). The results are summarized in Table 163.

Table 163: Acute toxicity (96 h) of pyraclostrobin to eastern oysters (*Crassostrea virginica*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0033**	**0.0055**	**0.0090**	**0.015**	**0.025**
Concentration [mg a.s./L] (mean measured)	--	--	0.0027	0.0041	0.0065	0.0128	0.0215
Mortality after 96 h [%]	5	0	0	0	0	5	10
Shell growth after 96 h [% of control]	--	109	100	117	70 *	52 *	35 *
Endpoints [mg pyraclostrobin/L] (mean measured)							
LC_{50} (96 h)	LC_{50} was > 0.0215						
EC_{50} (96 h)	0.0125 (95% confidence limits: 0.0092 - 0.0171)						
NOEC (96 h)	0.00406						

* Statistically significant difference compared to the pooled control / solvent control (Bonferroni's test).

Validity criteria according to EPA OPPTS 850.1025 (1996)	**Obtained in this study**
The mortality in the controls should not exceed 10 percent at the end of the test.	5% (control); 0% (solvent control)
The dissolved oxygen concentration should be at least 60 percent of air saturation throughout the test.	> 60% (5.5 – 8.5 mg/L)
If evidence of spawning is observed, the test should be repeated.	No spawning reported.
There should be evidence that the concentration of the substance being tested has been satisfactorily maintained over the test period. The concentration of the test substance should be measured: (1) In each chamber at time 0-h; (2) In each chamber at 96–h; (3) In at least one appropriate chamber whenever a malfunction is detected in any part of the test chemical delivery system.	Measurements in all test concentrations at test 0 h and 96 h; 72 - 89%; therefore, results are based on mean measured
Dissolved oxygen, temperature, salinity, and pH measurements should be made at the beginning and end of the test in each chamber.	Done
A minimum of 2 mm of new shell growth should be observed in control oysters (solvent and dilution water).	2.3 mm (control) 2.5 mm (solvent control)

Conclusions

In a flow-through acute toxicity study with eastern oysters (*Crassostrea virginica*), the LC_{50} was > 0.0215 mg a.s./L and the EC_{50} (96 h) for pyraclostrobin was 0.0125 mg/L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.00406 mg a.s./L.

The study is considered to be valid and reliable. It is relevant for classification purposes.

11.5.2.4 Acute toxicity to invertebrates – *Leptocheirus plumulosus*

Author:	Gaertner, K.
Title:	BAS 500 F: Whole sediment acute toxicity to a marine amphipod (*Lepto-cheirus plumulosus*)

Date:	15.01.2013
Doc ID:	68249; BASF Reg. Doc. No. 2013/7000055, Study No. 407438
Guidelines:	EPA 850.1740
GLP:	Yes
Validity:	Acceptable (based on US EPA criteria)
Previous evaluation:	Submitted for the purpose of renewal

Material and Methods

Test item:	Mixture of non-radiolabeled pyraclostrobin (BAS 500 F; Reg. no.: 304 428, batch no. COD-001236; purity: 99.02%) and 14C-radiolabeled pyraclostrobin (batch no. 579-6009; specific activity: 64.4 MBq/g; radiochemical purity: 99.4%; chemical purity: 100%).
Test species:	Marine amphipod (*Leptocheirus plumulosus*), 2 - 4 mm length; source: "Chesapeake Cultures", Hayes, Virginia, USA.
Test design:	Static system (10 days); 5 test concentrations plus a control and a solvent control, 8 replicates per test item concentration and per control group, 20 amphipods per replicate; assessment of survival after 10 days.
Endpoints:	NOEC and LC_{50}.
Test concentrations:	Control (dilution water), solvent control, 0.63, 1.3, 2.5, 5.0 and 10 mg a.s./kg dry sediment (nominal); corresponding to geometric mean measured TRR concentrations (14C-labeled pyraclostrobin equivalents) of 0.771, 1.51, 2.74, 5.05 and 11.3 mg a.s./kg dry sediment.
Test conditions:	1 L glass jars (17 cm height x 8.5 cm diameter) filled with 251.8 g natural marine sediment (27% sand, 55% silt, 18% clay, 6.4% organic matter), 600 mL dilution water (prepared by mixing a commercial sea salt mix to laboratory freshwater); salinity: 19.0 - 21.0‰; pH 7.6 - 8.5; oxygen content: 4.3 mg/L - 7.6 mg/L; water temperature: 24.3°C - 25.3°C; light intensity: 501 - 601 lux; photoperiod: 24 h light; continuous aeration.
Analytics:	Overlaying water, interstitial (pore) water and sediment were analyzed for total radioactive residues (TRR) using a liquid scintillation counting (LSC) method. The concentration of pyraclostrobin at the lowest treatment level was also measured in the overlying and interstitial water using an HPLC-method with UV-detection.
Statistics:	Descriptive statistics, Fisher's one-tailed exact test for determination of the NOEC value and for comparison of the dilution water control data and the solvent control data ($p = 0.05$).

Results and Discussion

Analytical measurements: Analytical verification of 14C-labeled pyraclostrobin concentrations in the sediment, the overlying water and the interstitial water was conducted in the controls and in each concentration at the beginning and the end of the test via LSC analysis. Mean measured concentrations of 14C-labeled pyraclostrobin TRR in the sediment were in a range between 117 and 125% of nominal concentrations at test initiation and between 89 and 120% of nominal at test termination. The geometric mean measured concentrations of 14C-labeled pyraclostrobin in samples taken from the nominal concentrations of 0.63, 1.3, 2.5, 5.0 and 10 mg a.s./kg dry sediment were determined to be 0.771, 1.51, 2.74, 5.05 and 11.3 mg a.s./kg dry sediment, respectively, which represent 101 to 122% of nominal concentrations. The geometric mean measured concentrations of 14C-labeled pyraclostrobin in the overlaying water samples were 0.000526, 0.00136, 0.00282, 0.00541 and 0.0115 mg TRR/L for the 0.63, 1.3, 2.5, 5.0 and 10 mg a.s./kg dry sediment samples, respectively. The respective geometric mean measured concentrations of 14C-labeled pyraclostrobin in the interstitial water samples were 0.00169, 0.00338, 0.00625, 0.0115 and 0.0229 mg TRR/L.

Additionally, the concentration of pyraclostrobin in the controls and at the lowest treatment level was measured in overlying and interstitial water at test initiation and test termination via HPLC/UV analysis. Measured

concentrations in overlaying water samples of two replicates of the 0.63 mg a.s./kg dry sediment treatment were 0.000128 and 0.000705 mg a.s./L at test initiation and 0.000342 and 0.0000492 mg a.s./L at test termination. In general, no residues of pyraclostrobin were detected in the control samples above the respective minimum quantifiable limits (MQL) in all measurements.

The following biological results are based on the geometric mean measured 14C-labeled pyraclostrobin sediment concentrations. The TRR concentrations were corrected for dry weight of sediment. The results of the chemical analysis are shown in Table 164 and Table 165 (extracted from study report).

Table 164: Analytical results – overlying water

Nominal Sediment Concentration (mg a.i./kg dry sediment)	Measured BAS 500 F Concentration as mg TRR/L		
	Day 0 [a]	Day 10 [b]	Geometric Mean Measured Concentration (Days 0-10)
0 (Control)	<MQL [c]	<MQL [d]	<MQL
	<MQL [c]	<MQL [d]	
0 (Vehicle Control)	<MQL [c]	<MQL [d]	<MQL
	<MQL [c]	<MQL [d]	
0.63	0.00180	0.00123	0.00169
	0.00203	0.00182	
1.3	0.00393	0.00262	0.00338
	0.00441	0.00287	
2.5	0.00775	0.00529	0.00625
	0.00811	0.00459	
5.0	0.149	0.00859	0.0115
	0.150	0.00920	
10	0.286	0.0199	0.0229
	0.275	0.0176	

[a] Measured from replicates M and N.
[b] Measured from replicates J and K.
[c] MQL = 0. 0000684 mg TRR/L.
[d] MQL = 0. 0000664 mg TRR/L.

Table 165: Analytical results - sediment

Nominal Sediment Concentration (mg a.i./kg dry sediment)	Measured TRR Concentration as mg BAS 500 F/kg dry sediment (Percent of Nominal)		
	Day 0 [a]	Day 10 [b]	Geometric Mean
0 (Control)	<MQL [c] <MQL [c]	<MQL [d] <MQL [d]	<MQL [d]
0 (Vehicle Control)	<MQL [c] <MQL [c]	<MQL [d] <MQL [d]	<MQL [d]
0.63	0.828 0.751	0.778 0.732	0.771 (122%)
1.3	1.70 1.40	1.49 1.46	1.51 (116%)
2.5	2.96 2.99	2.66 2.40	2.74 (110%)
5.0	4.57 7.26	4.12 4.75	5.05 (101%)
10	11.6 11.8	10.3 11.4	11.3 (113%)
Low Spike[e]	0.430 (96)	0.482 (106)	
High Spike[f]	12.0 (98)	12.6 (103)	

[a] Measured from replicates M and N.
[b] Measured from replicates J and K.
[c] MQL = 0.0192mg TRR/kg.
[d] MQL = 0.0160mg TRR/kg.
[e] Low Spike nominal concentration on Day 0 = 0.446 mg/Kg; on Day 10 = 0.456 mg/Kg.
[f] High Spike nominal concentration on Day 0 = 12.3 mg/Kg; on Day 10 = 12.2 mg/Kg.

Biological results: No statistically significant differences between the survival data in the dilution water control and the solvent control were detected (Fisher`s exact test; p = 0.05). The dilution control data were used for statistical evaluation of treatment related effects. After 10 days of exposure, the mean survival was 99 and 98 % in the dilution water control and the solvent control, respectively. Survival rates at the test item concentrations of 0.771, 1.51, 2.74, 5.05 and 11.3 mg a.s./kg dry sediment were 98, 100, 97, 25 and 0.6 %, respectively. Statistically significant differences compared to the dilution water control were observed at the two highest test concentrations (Fisher`s exact test; p = 0.05). The results are summarized in Table 166.

Table 166: Effect of pyraclostrobin on survival of *Leptocheirus plumulosus*

Concentration [mg a.s./kg dry sediment] (nominal)	**Control**	**Solvent control**	**0.63**	**1.3**	**2.5**	**5.0**	**10**
Concentration [mg a.s./kg dry sediment] (geometric mean measured)	--	--	0.771	1.51	2.74	5.05	11.3
Survival (10 d) [%]	99	98	98	100	97	25 *	0.6 *
Endpoints [mg a.s./kg dry sediment] (geometric mean measured)							
LC_{50} (10 d)	4.412 (95% confidence limits: 4.192 - 4.643)						
NOEC (10 d)	2.740						

* Statistically significantly difference compared to the dilution water control (Fisher`s exact test; p = 0.05).

Validity criteria according to EPA OPPTS 850.1740 (2016)	Obtained in this study
Dissolved oxygen concentration in overlaying water: ≥ 60% saturation	4.3 – 7.6 mg/L (60 – 105% saturation)
Overlaying water pH should be constant within ± 1 pH unit within a test vessel	7.6 – 8.5
Overlaying water salinity: 20 ± 3 ppt	19.0 – 21.0 ppt
Sediment pore water salinity between 0 - 33 ppt for *L. plumulosus*	27.0 – 29.5 ppt
Overlaying water TOC ≤ 2 mg/L	Not assessed *
Water temperature: 25 ± 2°C	– 25.2°C

* Note that the study was conducted before the release of the recent version of this guideline. The older version from 1996 did not include any recommendations on water TOC measurements.

Conclusions

In a 10-day static acute sediment test with *Leptocheirus plumulosus*, the LC_{50} of pyraclostrobin was determined to be 4.412 mg a.s./kg dry sediment based on mean measured concentrations. The NOEC was 2.740 mg a.s./kg dry sediment. One recent criterion of the US EPA guideline could not be verified. However, as all other criteria were met, this criterion was not included in the former guideline version and the test overall is plausible, the study can be considered as valid.

The study is considered reliable. It is relevant for classification purposes.

11.5.2.5 Acute toxicity to invertebrates – *Hyalella azteca*

Author:	Staggs, M.L.
Title:	BAS 500 F - 10-day toxicity test exposing freshwater amphipods (*Hyalella azteca*) to a test substance applied to water under static conditions
Date:	05.01.2018
Doc ID:	BASF Doc. No. 2017/7018168, Study No. 986.6315
Guidelines:	EPA 850.1735, OECD 219
GLP:	Yes
Validity:	Acceptable (based on US EPA criteria)
Previous evaluation:	Submitted for the purpose of renewal

Material and Methods

Test item:	Pyraclostrobin (BAS 500 F, Reg. No.: 304428), batch No.: COD-001236, purity: 99.02%
Test species:	Fresh water amphipod *Hyalella azteca*, 8 days old, source: Smithers Viscient laboratory cultures.
Test design:	Static system (10 days) with sediment; 5 test concentrations plus a water control; 9 replicates per test item concentration and for the control (4 replicates for biological response of test organisms and 5 replicates for chemical analysis); 20 amphipods per test vessel; daily assessment of survival and abnormal behaviour; determination of dry weight at test end.
Endpoints:	NOEC and LC_{50} / EC_{50} based on survival and growth.

Test concentrations:	Control (dilution water), nominal concentrations of 0.006, 0.010, 0.015, 0.025 and 0.040 mg a.s./L; corresponding to geometric mean measured concentrations of 0.0025, 0.0048, 0.0068, 0.012, and 0.019 mg a.s./L.
Test conditions:	1.0 L glass beakers; water to sediment ratio 10:1 (approx. 935 mL overlaying water and approx. 85 mL sediment); dilution water: laboratory well water; test sediment: Smithers Viscient sediment (batch no. 060917A); (approx..; pH: 6.6 - 7.3; oxygen content: 6.2 - 8.2 mg/L (72 - 95% saturation); total hardness: 40 - 68 mg $CaCO_3$/L; total alkalinity: 18 - 32 mg $CaCO_3$/L; conductivity: 510 - 590 µS/cm; ammonia: 0.33 - 3.2 mg nitrogen/L; water temperature: 23 - 24°C; photoperiod: 16 hours light and 8 hours darkness; light intensity: 580 – 880 lux; feeding: 3 mL of commercial daphnia food (YCT; yeast, cereal leaves, Tetramin) daily.
Analytics:	Analytical verification of test item concentrations was conducted using an LC-method with MS/MS detection.
Statistics:	Descriptive statistics, Dunnett's T3 multiple comparison test for comparison of control and treatment data for survival ($p \leq 0.05$), Dunnett's multiple comparison test for comparison of control and treatment data for growth ($p \leq 0.05$).

Results and Discussion

Analytical measurements: Analytical verification of test item concentration in overlying water was conducted in each concentration at test initiation, after one day and at test termination. Initial measured concentrations of pyraclostrobin in overlying water were between 126.7 and 140.0% of nominal concentrations. On day 1 after test start, the measured test item concentrations in overlying water were between 55.3 and 72.0% of nominal concentrations. At test termination, the measured test item concentrations in overlying water were between 8.8 and 13.3% of nominal concentrations.

Analytical verification of test item concentration in sediment was conducted in each concentration day 1 after test start and at test termination. On day 1 after test start, the measured test item concentrations in sediment were 6.0 – 28.0 µg/kg sediment dry weight. At test termination, the measured test item concentrations in sediment were 27.0 – 200.0 µg/kg sediment dry weight. The biological results are based on geometric mean measured concentrations in overlying water.

The results of the chemical analysis are shown in Table 167 and Table 168 (extracted from study report):

Table 167: Analytical results – overlying water

Nominal Concentration (µg/L)	Measured Overlying Water Concentration (µg/L)			Geometric Mean Measured Concentration	Percent of Nominal
	Day 0[a]	Day 1[b]	Day 10[c]		
Control	< 0.050[d]	< 0.050	< 0.050	NA[e]	NA
6.0	7.9	3.8	0.53	2.5	42
10	14	6.7	1.2	4.8	48
15	19	8.3	2.0	6.8	45
25	35	18	2.5	12	46
40	52	27	4.9	19	47
QC[f]#1 0.100	0.124 (124)[g]	0.112 (112)	0.0832 (83.2)		
QC#2 4.00	4.48 (112)	4.01 (100)	3.88 (96.9)		
QC#3 40.0	42.6 (107)	40.7 (102)	37.5 (93.7)		

a Analytical samples were removed from replicate E.
b Analytical samples were removed from replicate G.
c Analytical samples were removed from replicate H.
d Concentrations expressed as less than values were below the method detection limit (MDL). The MDL is dependent upon the lowest concentration calibration standard and the dilution factor of the controls.
e NA = Not Applicable
f QC = Quality Control sample. Measured concentration of each QC sample is presented with the percent recovery in parentheses. Percent recoveries were calculated using the original unrounded results and not the rounded numbers presented in this table.
g Analytical result for this QC sample was outside of the acceptance criteria (i.e., 80.0 to 120%, Appendix 3). See additional discussion below.

Table 168: Analytical results - sediment

Nominal Concentration (µg/L)	Measured Sediment Concentration (µg/kg sediment dry weight)		
	Day 1[a]	Day 10[b]	Geometric Mean Measured Concentration (SD)[c]
Control	< 0.50[d]	< 0.50	NA[e] (NA)
6.0	6.0	27	13 (15)
10	6.2	44	17 (27)
15	28	59	40 (22)
25	15	120	42 (73)
40	28	200	75 (120)
QC[f]#1 0.750	0.749 (99.9)	0.991 (132)[g]	
QC#2 10.0	9.15 (91.5)	12.0 (120)	
QC#3 1000	949 (94.9)	844 (84.4)	

[a] Analytical samples were removed from replicate F.
[b] Analytical samples were removed from replicate H.
[c] Mean measured concentration and standard deviation (SD) values were calculated using the actual analytical results and not the rounded values (two significant figures) presented in this table.
[d] Concentrations expressed as less than values were below the method detection limit (MDL). The MDL is dependent upon the lowest concentration calibration standard and the dilution factor of the controls.
[e] NA = Not Applicable
[f] QC = Quality Control sample, concentrations are in µg/kg. Measured concentration of each QC sample is presented with the percent recovery in parentheses. Percent recoveries were calculated using the original unrounded results and not the rounded numbers presented in this table.
[g] Analytical result for this QC sample was outside of the acceptance criteria (i.e., 70.0 to 120%, Appendix 4). See additional discussion below.

Biological results: After 10 days of exposure, the mean survival in the control was 98%. Survival rates at the test item concentrations ranged from 95 to 98%. No statistically significant effects on survival was observed at any treatment levels tested compared to the control (Dunnett's T3 Multiple Comparison Test, $\alpha \leq 0.05$). A statistically significant reduction in growth compared to the control was observed at the highest test item concentration of 0.019 mg a.s./L (Dunnett's Multiple Comparison Test, $\alpha \leq 0.05$). The results are summarized in Table 169.

Table 169: Effect of pyraclostrobin on survival and growth of *Hyalella azteca*

Concentration [mg a.s./L] (nominal)	**Control**	**0.006**	**0.010**	**0.015**	**0.025**	**0.040**
Concentration [mg a.s./L] (geometric mean measured)	-	0.0025	0.0048	0.0068	0.012	0.019
10-day mean survival (SD) [%]	98 (3)	96 (3)	96 (5)	98 (3)	95 (0)	96 (3)
10-day mean amphipod dry weight (SD) [mg]	0.14 (0.025)	0.15 (0.0059)	0.16 (0.013)	0.14 (0.0068)	0.14 (0.0051)	0.12 * (0.0078)
Endpoints [mg BAS 500 F/L] (geometric mean measured)						
LC_{50} (10 d) survival	> 0.019					

EC_{50} (10 d) growth	> 0.019
NOEC (10 d) survival	≥ 0.019
NOEC (10 d) growth	0.012

* Significantly reduced compared to the negative control, based on Dunnett's Multiple Comparison Test, $\alpha \leq 0.05$.
SD = Standard Deviation

Validity criteria according to OCSPP Guideline 850.1735	Obtained in this study
Control(s) mortality ≤ 20%	2%
Animals were randomly assigned to the test vessels	yes
A negative control was included in the test	yes
Test vessels contained equal amounts of water and sediment	yes
A 2.5x increase in dry weight in the control(s)	Dry weight per amphipod in the negative control increased by 12x

Conclusions

In a 10-day static test with *Hyalella azteca* the LC_{50} / EC_{50} of pyraclostrobin for both survival and growth was determined to be > 0.019 mg a.s./L based on geometric mean measured concentrations. The NOEC (10 d) values for survival and growth were ≥ 0.019 mg a.s./L and 0.012 mg a.s./L, respectively (geometric mean measured).

It has to be highlighted that there were some deviations from the US EPA guideline, 20 Organisms per replicate were used instead of recommended 10 per replicate and the water and sediment volume were 85 mL sediment to 850 mL water instead of 100 mL sediment and 175 mL water. As the validity criteria were met, the test conditions were fulfilled and the results are plausible, it can be concluded that these deviations had no impact on the outcome of the study.

The validity criteria of US EPA 850.1735 were met and the study is considered as valid and reliable. It is relevant for classification purposes.

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

11.5.3.1 Toxicity to algae – *Pseudokirchneriella subcapitata*

Author:	Dohmen, G.P.
Title:	Effect of BAS 500 F on the growth of the green alga *Pseudokirchneriella subcapitata*
Date:	15.09.1999
Doc ID:	35803; BASF Reg.Doc. ID 1999/11020
Guidelines:	OECD 201
GLP:	Yes
Validity:	Acceptable. The validity criteria of the OECD 201 adopted 2006 were checked and are fulfilled with some restriction as the section-by-section coefficient of variation could only be evaluated for day 2-3 and 3-4 but not for day 0-1 and 1-2 as no data are available for day 1.
Previous evaluation:	In initial DAR (2001)

Author	Hoffmann, F.
Title:	Effect of BAS 500 F on the growth of the green alga *Pseudokirchneriella subcapitata* - Additional calculation of the inhibition values for growth rate and yield data after a test period of 72 h
Date:	26.02.2009
Doc ID:	BASF DocID 2009/1037148
Guidelines:	OECD 201
GLP:	Not applicable (complementary information to original study
Validity:	Additional information, original study acceptable
Previous evaluation:	Submitted for the purpose of renewal

Material and Methods

Test item:	BAS 500 F (batch J.-Nr. 27822/191/c (Tox III/1)); purity: 97.09 %
Test species:	Green alga (*Pseudokirchneriella subcapitata*), SAG 61.81
Test design:	Static system (96 hours); 3 x 10^3 algae cells/mL at test initiation. Eight test item concentrations and a negative (water) control; five replicates per treatment and ten replicates for control; assessment of growth on day 2, 3 and 4.
Endpoints:	E_rC_{10}, E_rC_{50}, E_bC_{10}, E_bC_{50}, morphological effects after 96 h; additional calculation of E_rC_{10}, E_rC_{50}, E_yC_{10} and E_yC_{50} after 72 h.
Test concentrations:	Control, 0.008, 0.016, 0.031, 0.063, 0.125, 0.250, 0.5, 1.0 mg as/L (nominal)
Test conditions:	Erlenmeyer flasks (100 mL), test volume: 60 mL inoculated with an initial algal concentration of 3 x 10^3 cells/mL, in a temperature-controlled room; temperature: 22 +/- 1 °C; constant shaking of the flasks at 135 rpm; pH 7.98 – 8.0; illumination: constant approx. 8000 lux.
Analytics:	HPLC
Statistics:	Probit Analysis

Results and Discussion

Analytical measurements: Analytical verification of test item concentration was conducted in each test concentration. The analyzed contents ranged from 82.0% to 94.7% of nominal at test initiation and from 77.3% to 91.5% of nominal at test termination. The analytical results are presented in Table 170.

Table 170: Analytical results

Nominal concentration (mg/L)	Mean measured concentration (mg/L) 0 h	% of nominal	Mean measured concentration (mg/L) 96 h	% of nominal
Control	0	-	-	-
0.008	0.0076	94.7	0.0071	88.8
0.016	0.0145	90.7	0.0130	81.1
0.031	0.0284	91.6	0.0259	83.5
0.063	0.0588	93.4	0.0556	88.2
0.125	0.1119	89.6	0.1134	90.7
0.250	0.2328	93.1	0.1932	77.3

0.500	0.4383	87.7	0.4577	91.5
1.0	0.8201	82.0	0.8648	86.5

Biological results: BAS 500 F caused no morphological effects on the algae. The biological results are summarized in Table 171.

Table 171: Effect (96 h) of pyraclostrobin on the growth of the freshwater green alga *Pseudokirchneriella subcapitata*

Concentration [mg a.s./L] (nominal)	**0.008**	**0.016**	**0.031**	**0.063**	**0.125**	**0.250**	**0.500**	**1.0**
Concentration [mg a.s./L] (mean measured)	**0.007**	**0.014**	**0.027**	**0.057**	**0.113**	**0.213**	**0.448**	**0.843**
Inhibition in 96 h [%] (based on growth rate)	-1.1	3.3	6.8	6.5	12.2	15.6	27.6	49.5
Inhibition in 96 h [%] (based on biomass)	0.2	12.5	28.4	26.1	42.6	51.9	68.2	87.2
Endpoints [mg pyraclostrobin/L] (mean measured)								
E_rC_{50} (96 h)	> 0.843 extrapolated: 1.282 (95% confidence limits: 1.110 - 1.482)							
E_rC_{10} (96 h)	0.078 (95% confidence limits: 0.071 - 0.087)							
E_bC_{50} (96 h)	0.152 (95% confidence limits: 0.143 - 0.162)							
E_bC_{10} (96 h)	0.014 (95% confidence limits: 0.013 - 0.015)							
E_rC_{50} (72 h)	> 0.843							
E_rC_{10} (72 h)	0.071 (95% confidence limits: 0.064 - 0.080)							
E_yC_{50} (72 h)	0.148 (95% confidence limits: 0.140 - 0.158)							
E_yC_{10} (72 h)	0.013 (95% confidence limits: 0.012 - 0.015)							

Validity criteria according to OECD TG 201 (2011)	**Obtained in this study**
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92/day (for species in Annex 2 of OECD TG 201)	44-fold increase
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%. *	48 -72 h: 4.6% 48 -96 h: 9.8%
The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. For other less frequently tested species, the value should not exceed 10%. *	48 – 96 h: 2.6% 48 – 72 h: 4.6%

[#] Evaluations are based on data for day 2-3 and 3-4 but not for day 0-1 and 1-2 as no data are available for day 1.

Conclusions

In a static 96-hour study with the green algae *Psudokirchneriella subcapitata* the ErC_{50} (96 h) of BAS 500-14 was > 0.843 mg a.s./L based on mean measured concentrations. The EbC_{50} (96 h) was determined to be 0.152 mg a.s./L (mean measured). Based on the additional calculation the 72-hour ErC_{50} was > 0.843 mg a.s./L based on mean measured concentrations. The 72-hour EyC_{50} was calculated to be 0.148 mg a.s./L (mean

measured).

It is noted that one of the validity criteria, i.e. the section-by-section coefficient of variation could not fully be verified as an evaluation is not possible for day 0-1 and 1-2. However as the criterion is far below the trigger of ≤ 35% for day 2-3 and 3-4, it can be assumed that day 0-1 and 1-2 would also meet the criterion. Beside that all other criteria were fully met.

The overall study is considered valid and reliable. It is relevant for classification purposes.

11.5.3.2 Toxicity to algae – *Navicula pelliculosa*

Author:	Boeri, R.L. et al.
Title:	Growth and Reproduction Toxicity Test with BAS 500F and the Freshwater Alga, *Navicula pelliculosa*
Date:	13.03.2000
Doc ID:	1321-BA; BASF Reg.Doc. 2000\5046, BASF Study Number 97127
Guidelines:	EPA 123-2, EPA 850.5400
GLP:	Yes
Validity:	Acceptable (based on US EPA Guideline and OECD 201; deviations: initial cell number only 3000 cells/mL instead of 10000 cells/mL, only 3 replicates instead of at least 4; deviations were regarded to have no impact on the outcome of the study)
Previous evaluation:	Submitted for the purpose of renewal

Material and Methods

Test item:	BAS 500 F (batch no. 27882/191/C); purity: 97.09 %
Test species:	Freshwater diatom, *Navicula pelliculosa*, strain UTEX 664, stock originally obtained from the "Culture Collection of Algae", University of Texas, Austin, USA.
Test design:	Static system; test duration 120 hours; 5 test concentrations plus a dilution water control and a solvent control with 3 replicates for each; daily assessment of growth.
Endpoints:	EC50 with respect to biomass and growth rate after exposure over 72 and 120 hours.
Test concentrations:	Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.0013, 0.0025, 0.0050, 0.010 and 0.020 mg pyraclostrobin/L (nominal), corresponding to initial measured concentrations of 0.00118, 0.00240, 0.00486, 0.00908 and 0.0184 mg a.s./L.
Test conditions:	250 mL glass flasks; test volume 50 mL; sterile enriched medium supplemented with 0.2 g/L Na2SiO3 x 9 H2O; pH 7.4 at test initiation and pH 7.3 - 7.6 at test termination; temperature: 23.2°C - 23.7°C; initial cell densities 3 x 103 cells/mL; continuous light at 4100 - 4500 lux; constant shaking at 100 rpm.
Analytics:	HPLC method with UV detection
Statistics:	Descriptive statistics; t-test (α = 0.05) for comparison of cell densities and growth rate data in the control and the solvent control; weighted least squares non-linear regression analysis for determination of ECx values.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of pyraclostrobin ranged from 91 to 97% of nominal concentrations at test initiation and from 50 to 52% of nominal at test termination. The biological results were based on initial measured concentrations. The results of the chemical analysis are shown in Table 172 (extracted from study report):

Table 172: Analytical results

Nominal Concentration of BAS 500 F (µg/L)	Measured Concentration of BAS 500 F (µg/L)			
	0 Hour	% Recovery	120 Hour	% Recovery
Test Media				
0 (control)	ND[1]	---	ND	---
0 (solvent control)	ND	---	ND	---
1.3	1.18	91	ND[3]	---
2.5	2.40	96	1.27	51
5.0	4.86	97	2.62	52
10	9.08	91	5.17	52
20	18.4	92	9.95	50
Blank				
0	ND	---	ND	---
Matrix Spike[2]				
5.0	--- ---	---	4.15 3.82	83 76
Laboratory Control Spike[2]				
5.0	4.46	89	4.38	88

[1] ND = none detected at or above the limit of quantitation of 0.50 µg/L.
[2] Matrix spike samples and laboratory control samples were prepared with the test substance used to formulate the test concentrations (purity = 97.09%).
[3] Analysis resulted in a value of 0.48 µg/L, which is greater than the LOD but less than the LOQ.

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. Statistically significant differences of the cell numbers and average specific growth rates compared to the pooled control were observed at the four highest test item concentrations after exposure over 120 hours (Bonferroni's test; $\alpha = 0.05$). The effects on algal growth are summarized in Table 173.

Table 173: Effect of pyraclostrobin on biomass development and growth of the freshwater diatom *Navicula pelliculosa*

Concentration [mg a.s./L] (nominal)	**Water control**	**Solvent control**	**0.0013**	**0.0025**	**0.0050**	**0.010**	**0.020**
Concentration [mg a.s./L] (initial measured)	--	--	0.00118	0.00240	0.00486	0.00908	0.0184
Mean cell density (72 h) [% of water control]	--	108	78	33	26	19	19
Growth rate (72 h) [% of water control]	--	102	92	70	62	54	54
Mean cell density (120 h) [% of water control]	--	80	82	16	12	13	12

Concentration [mg a.s./L] (nominal)	**Water control**	**Solvent control**	**0.0013**	**0.0025**	**0.0050**	**0.010**	**0.020**
Concentration [mg a.s./L] (initial measured)	--	--	0.00118	0.00240	0.00486	0.00908	0.0184
Growth rate (120 h) [% of water control]	--	96	96	72	68	70	68
Endpoints [mg pyraclostrobin/L] (initial measured)							
E_rC_{50} (72 h)	0.0158 (95% confidence limits: 0.009 - > 0.0184)						
E_rC_{25} (72 h)	0.0019 (95% confidence limits: < 0.00118 - 0.0147)						
E_bC_{50} (72 h)	0.00165 (95% confidence limits: < 0.00118 - 0.00314)						
E_bC_{25} (72 h)	< 0.00118						
E_rC_{50} (96 h)	> 0.0184						
E_rC_{25} (96 h)	0.00128 (95% confidence limits: < 0.00118 - 0.00214)						
E_bC_{50} (96 h)	< 0.00118						
E_bC_{25} (96 h)	< 0.00118						
E_rC_{50} (120 h)	> 0.0184						
E_rC_{25} (120 h)	0.0058						
E_bC_{50} (120 h)	0.00150 (95% confidence limits: < 0.00118 - 0.00383)						
E_bC_{25} (120 h)	< 0.00118 (95% confidence limits: 0.00243 - 0.0138)						
NOE_rC/NOE_bC (120 h)	0.00118						

Validity criteria according to OECD TG 201 (2011)	**Obtained in this study**
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92/day (for species in Annex 2 of OECD TG 201)	36-fold increase (72 h) 907-fold increase (120 h)
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%.	13.9% (72 h)
The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. For other less frequently tested species, the value should not exceed 10%.	1.0%

Conclusions

In a 120-hour algae toxicity test with *Navicula pelliculosa*, the E_rC_{50} (120 h) for pyraclostrobin was determined to be > 0.0184 mg a.s./L. After 72 hours of exposure, the respective E_rC_{50} was determined to be 0.0158 mg a.s./L.

In the study report the endpoints are based on initial measured concentrations as the concentrations at test start were > 90 % of nominal while at the end of the test only 50 to 52 % of nominal were measured. A laboratory control spike (without algae) was performed with 0.005 mg a.s./L, representing the medium concentration of the test concentrations. After 0 hours the measured concentration was 0.00446 mg a.s./L i.e. 89 % of nominal. After 120 hours the measured concentration was 0.00438 mg a.s./L i.e. 88 % of nominal. Based on this result there was nearly no loss of test substance and the concentrations were > 80 % of nominal during a period of 5 days. But the maintenance of the test substance in the control spike was only tested for this single concentration

hence it is not fully clear if the same accounts for the other concentrations, especially the lower ones. From the study report it looks as this control spike was only tested once, i.e. there was no repetition. Thus it is not clear how reliable this result is. It has to be considered that all E_bC_{50} values (for 72, 96 and 120 hours) and the NOEC were below the test concentration of 0.005 mg a.s./L. Only the ErC_{50} values for 72, 96 and 120 hours were above the test concentration of 0.005 mg a.s./L. Although the loss of test substance was nearly equal in all treatments until the end of the study, i.e. after three days for all concentrations there were 50-52 % of nominal left, we are not sure that this single control spike with 0.005 mg a.s./L is sufficient to verify that the maintenance of the test substance without algae would be at most +/- 20 % from nominal for all concentrations.

And even if one considers this single laboratory control spike as sufficient to verify that the test substance was adsorbed by the algae cells the question is what kind of consequence this has? It is not known if the adsorbed test substance still can have an effect or not. Thus the conservative approach is to base the endpoints on mean measured concentrations is justified for precautionary reasons. As analytical measurements were only performed at day 0 and 5 it is not possible to calculate mean measured concentrations for the, according to US EPA Guideline relevant 96-hour endpoint, but only for the 5 day test period. However as it can be assumed that test item concentrations are higher at day four compared to day five the approach to consider the mean measured concentrations day 0 – 5 (i.e. a mean of 69.5% from nominal) for endpoint derivation can be considered as conservative. Based on mean measured concentrations the 96-h E_rC_{50} was > 0.013 mg a.s./L and the E_bC_{50} was < 0.0008 mg a.s./L. The 72-h E_rC_{50} would be approximately 0.011 mg a.s./L and the E_bC_{50} would be about 0.001 mg a.s./L based on mean measured concentrations. The 72-h ErC_{50} based on mean measured concentrations was considered as relevant endpoint for the risk assessment. Although a newer study with *N. pelliculosa* performed according to OECD 201 was provided, the study is still considered as relevant. From the newer study a clearly higher endpoint was derived. Both studies are valid, but it is unclear why the studies result in so different endpoints. For precautionary reaons the lower endpoint is considered in further assessment.

The study is considered valid and reliable with restrictions. It is relevant for classification purposes.

11.5.3.3 Toxicity to algae – *Anabaena flos-aquae*

Author:	Boeri, R.L. et al.
Title:	Growth and reproduction toxicity test with BAS 500 F and the freshwater alga, *Anabaena flos-aquae*
Date:	22.02.2000
Doc ID:	1222-BA; BASF Reg.Doc. 200\5036, BASF Study Number 97128
Guidelines:	EPA 123-2, EPA 850.5400
GLP:	Yes
Validity:	Not acceptable (US EPA criteria are fulfilled but OECD 201 criteria not)
Previous evaluation:	Submitted for the purpose of renewal

Material and Methods

Test item:	BAS 500 F (batch no. 27882/191/C); purity: 97.09 %
Test species:	Freshwater blue-green alga, *Anabaena flos-aquae*; stock originally obtained from the University of Texas, Austin, USA; stock was maintained at test conditions for more than 14 days before the test.
Test design:	Static system (120 hours); 5 test concentrations plus a dilution water control and a solvent control with 3 replicates for each; daily assessment of growth.

Endpoints:	EC50 with respect to biomass and growth rate after exposure over 96 hours and 120 hours (72 h EC50 values could not be calculated because sufficient growth had not yet occurred during the first 72 hours in any of the test groups to allow the calculation to be made).
Test concentrations:	Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.13, 0.25, 0.50, 1.0 and 2.0 mg pyraclostrobin/L (nominal), corresponding to mean measured concentrations of 0.118, 0.219, 0.466, 0.911 and 1.78 mg a.s./L.
Test conditions:	250 mL glass flasks; test volume: 50 mL; sterile enriched medium; pH 7.4 - 7.6 at test initiation and pH 7.8 - 8.0 at test termination; temperature: 23.7°C - 24.5°C; initial cell densities: 3 x 103 cells/mL; continuous light at about 2000 lux, continuous shaking at 100 rpm.
Analytics:	HPLC method with UV detection
Statistics:	Descriptive statistics, t-test (α = 0.05) for comparison of cell densities and growth rate in the control and solvent control; Bonferroni's test for determination of the NOEC values (α = 0.05).

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured concentrations of pyraclostrobin ranged from 96.4 to 102.8% of nominal concentrations at test initiation and from 78.4 to 83.6% of nominal at test termination. The following biological results are based on mean measured concentrations. The results of the chemical analysis are shown in Table 174 (extracted from study report):

Table 174: Analytical results

Nominal Concentration of BAS 500 F (mg/L)	Measured Concentration of BAS 500 F (mg/L)			Percent of Nominal
	0 Hour	120 Hour	Mean	
Test Media				
0 (control)	ND[1]	ND	ND	--
0 (solvent control)	ND	ND	ND	--
0.13	0.129	0.106	0.118	91
0.25	0.241	0.196	0.219	88
0.50	0.514	0.418	0.466	93
1.0	1.00	0.821	0.911	91
2.0	1.98	1.57	1.78	89
Blank				
0	ND	ND	--	--
Matrix Spike Sample[2]				
0.50	--	0.522		
	--	0.534	0.528	106
Laboratory Control Sample				
0.50	0.526	0.537	0.532	106

[1] ND = none detected at or above the limit of quantitation of 0.00050 mg/L.
[2] Matrix spike samples and laboratory control samples were prepared with the test substance used to formulate the test concentrations (purity = 97.09%).

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; α = 0.05). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. No statistically significant differences compared to the pooled control were observed at any test item concentrations after exposure over 120 hours (Bonferroni's

test; α = 0.05). EC50 values for 72 h could not be calculated because sufficient growth had not yet occurred during the first 72 hours in any of the test groups to allow the calculation to be made. The effects on algal growth are summarized in Table 175.

Table 175: Effect of pyraclostrobin on biomass development and growth of the blue-green alga *Anabaena flos-aquae*

Concentration [mg a.s./L] (nominal)	**Water control**	**Solvent control**	**0.13**	**0.25**	**0.50**	**1.0**	**2.0**
Concentration [mg a.s./L] (mean measured)	--	--	**0.118**	**0.219**	**0.466**	**0.911**	**1.78**
Mean cell density (96 h) [% of water control]	--	53	58	38	42	24	<14
Growth rate (96 h) [% of water control]	--	82	85	70	73	58	39
Mean cell density (120 h) [% of water control]	--	93	91	104	99	86	101
Growth rate (120 h) [% of water control]	--	100	98	102	100	98	100
Endpoints [mg pyraclostrobin/L] (mean measured)							
E_rC_{50} (96 h)	1.41 (95% confidence limits: 1.04 - > 1.78)						
E_rC_{25} (96 h)	0.455 (95% confidence limts: 0.275 – 0.752)						
E_bC_{50} (96 h)	0.367 (95% confidence limits: 0.184 - 0.734)						
E_bC_{25} (96 h)	< 0.118						
E_rC_{50} (120 h)	> 1.78						
E_rC_{25} 120 h)	> 1.78						
E_bC_{50} (120 h)	> 1.78						
E_bC_{25} (120 h)	> 1.78						
NOE_rC/NOE_bC (120 h)	≥ 1.78						

Validity criteria according to OECD TG 201 (2011)	**Obtained in this study**
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92/day (for species in Annex 2 of OECD TG 201)	<4-fold increase (72 h)
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%.	Evaluation not possible *
The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. For other less frequently tested species, the value should not exceed 10%.	20% (72 h)

* No exact cell densities are given *(i.e.* values are given as <10000 cells/mL) for the 24, 48 and 72 h measurements; thus, this validity criterion cannot be evaluated.

Conclusions

In a 120-hour algae toxicity test with *Anabaena flos-aquae*, the E_rC_{50} (120 h) for pyraclostrobin was determined to be > 1.78 mg a.s./L based on mean measured concentrations. After 96 hours of exposure, the respective E_rC_{50} value was determined to be 1.41 mg a.s./L.

The test was performed according to US EPA 850.5400 and was acceptable according to this guideline. However, *Anabaena flos-aquae* is also a test species considered by OECD guideline 201 and the validity criteria of the OECD guideline were not met. Hence it is agreed that the study should not be considered as valid any more.

The study is considered not reliable. It is not relevant for classification purposes.

11.5.3.4 Toxicity to algae – *Skeletonema costatum*

Author:	Boeri, R.L. et al.
Title:	Growth and reproduction toxicity test with BAS 500F and the marine alga, *Skeletonema costatum*
Date:	22.02.2000
Doc ID:	1223-BA; BASF Reg.Doc. 2000/5035, BASF Study Number 97126
Guidelines:	EPA 123-2, EPA 850.5400
GLP:	Yes
Validity:	Acceptable (based on US EPA Guideline)
Previous evaluation:	Submitted for the purpose of renewal

Material and Methods

Test item:	BAS 500 F (batch no. 27882/191/C); purity: 97.09 %
Test species:	Marine diatom, *Skeletonema costatum*, strain UTEX LB 2308, in-house culture; stock originally obtained from the "Culture Collection of Algae", University of Texas, Austin, USA.
Test design:	Static system (120 hours); 5 test concentrations plus a dilution water control and a solvent control with 3 replicates for each; daily assessment of growth.
Endpoints:	EC_{50} with respect to biomass and growth rate after exposure over 72 hours and 120 hours.
Test concentrations:	Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.013, 0.025, 0.050, 0.10 and 0.20 mg pyraclostrobin/L (nominal), corresponding to initial measured concentrations of 0.00973, 0.0194, 0.0381, 0.0816 and 0.159 mg a.s./L.
Test conditions:	250 mL glass flasks; test volume: 50 mL; enriched marine media; pH 8.0 at test initiation and pH 8.3 - 9.6 at test termination; temperature: 19.2°C - 21.1°C; initial cell densities: 1 x 10^3 cells/mL; photoperiod: 16 hours light : 8 hours dark, light intensity: 3900 - 4000 lux, continuous shaking at 100 rpm.
Analytics:	HPLC method with UV detection
Statistics:	Descriptive statistics, t-test ($\alpha = 0.05$) for comparison of cell densities and growth rate in the control and the solvent control; weighted least squares non-linear regression estimation procedure for determination of EC_x values, Bonferroni's test ($\alpha = 0.05$) for determination of the NOEC (120 h) values.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. At test initiation, mean measured concentrations of pyraclostrobin ranged from 75 to 82% of nominal concentrations. At test termination mean measured concentrations of pyraclostrobin were between 15 and 66% of nominal. The following biological results are based on initial measured concentrations. The results of the chemical analysis are shown in Table 176 (extracted from study report)

Table 176: Analytical results

Nominal Concentration of BAS 500 F (µg/L)	Measured Concentration of BAS 500 F (µg/L)			
	0 Hour	% Recovery	120 Hour	% Recovery
Test Media				
0 (control)	ND[1]	---	ND	---
0 (solvent control)	ND	---	ND	---
13	9.73	75	ND	---
25	19.4	78	3.82	15
50	38.1	76	11.6	23
100	81.6	82	40.1	40
200	159	80	131	66
Blank				
0	ND	---	ND	---
Matrix Spike[2]				
50	---		36.8	74
	---	---	36.2	72
Laboratory Control Spike[2]				
50	40.6	81	39.4	79

[1] ND = none detected at or above the limit of quantitation of 0.50 µg/L.
[2] Matrix spike samples and laboratory control samples were prepared with the test substance used to formulate the test concentrations (purity = 97.09%).

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. After 120 hours statistically significant differences compared to the pooled control were observed at the three and four highest test item concentrations for growth rate and mean cell density, respectively (Bonferroni's t-test; $\alpha = 0.05$). The effects on algal growth are summarized in Table 177.

Table 177: Effect of pyraclostrobin on biomass development and growth of the marine diatom *Skeletonema costatum*

Concentration [mg a.s./L] (nominal)	**Water Control**	**Solvent control**	**0.013**	**0.025**	**0.050**	**0.10**	**0.20**
Concentration [mg a.s./L] (initial measured)	--	--	0.00973	0.0194	0.0381	0.0816	0.159
Mean cell density (72 h) [% of water control]	--	104	51	25	16	13	6
Growth rate (72 h) [% of water control]	--	100	85	71	62	59	41
Mean cell density (120 h) [% of water control]	--	98	99	86	59	51	15
Growth rate (120 h) [% of water control]	--	98	98	96	89	87	65
Endpoints [mg pyraclostrobin/L] (initial measured)							
E_rC_{50} (72 h)	0.0962 (95% confidence limits: 0.0716 - 0.129)						
E_rC_{25} (72 h)	0.0178 (95% confidence limits: 0.0108 – 0.0292)						

Concentration [mg a.s./L] (nominal)	**Water Control**	**Solvent control**	**0.013**	**0.025**	**0.050**	**0.10**	**0.20**
Concentration [mg a.s./L] (initial measured)	--	--	0.00973	0.0194	0.0381	0.0816	0.159
E_bC_{50} (72 h)	< 0.00973						
E_bC_{25} (72 h)	< 0.00973						
E_rC_{50} (96 h)	> 0.159						
E_rC_{25} (96 h)	0.0422 (95% confidence limits: 0.0232 – 0.0766)						
E_bC_{50} (96 h)	0.0154 (95% confidence limits: < 0.00973 – 0.0275)						
E_bC_{25} (96 h)	< 0.00973						
E_rC_{50} (120 h)	> 0.159						
E_rC_{50} (120 h)	0.119 (95% confidence limits: 0.100 – 0.142)						
E_bC_{50} (120 h)	0.0647 (95% confidence limits: 0.0498 - 0.0840)						
E_bC_{50} (120 h)	0.0324 (95% confidence limits: 0.0211 – 0.0497)						
NOE_rC (120 h)	0.0194						
NOE_bC (120 h)	0.00973						

Validity criteria according to OECD TG 201 (2011)	**Obtained in this study**
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92/day (for species in Annex 2 of OECD TG 201)	131-fold increase (72 h)
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%.	**35.5% (72 h)**
The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with Pseudokirchneriella subcapitata and Desmodesmus subspicatus. For other less frequently tested species, the value should not exceed 10%.	4.4% (72 h)

Failure in terms of meeting one validity criterion is marked in **bold.**

Conclusions

In a 120-h algae test with *Skeletonema costatum*, the E_rC_{50} (120 h) of pyraclostrobin was determined to be > 0.159 mg a.s./L based on initial measured concentrations. After 72 hours of exposure, the respective E_rC_{50} value was determined to be 0.0962 mg a.s./L.

One criterion according to OECD 201 (mean coefficient of variation for section-by-section specific growth rate) is slightly exceeded at 72 h. However, it has to be highlighted that *Skeletonema costatum* is a marine species which does not present a regular OECD 201 test species thus it is not clear if the validity criteria can fully be transferred to this species. CVs were even higher for the growth rates after 96 and 120 hours, but according to OECD 201 the 72-hour rates are the ones to be taken into account. As the section-by-section growth rate CV was nearly met and all other criteria were fully met (according to OECD 201 and US EPA 850.4500) the study is considered as valid.

In the study report the endpoints are based on initial measured concentrations as the concentrations at the beginning of the test were measured to be < 80 % of nominal for some treatments. At the end of the test only 15 to 66 % of nominal were measured, hence the endpoints should be based on mean measured concentrations. As analytical measurements were only performed at day 0 and 5 it is not possible to calculate mean measured concentrations for the, according to US EPA Guideline relevant 96-hour endpoint, but only for the 5 day test

period. However as it can be assumed that test item concentrations are higher at day four compared to day five the approach to consider the mean measured concentrations day 0 – 5 (i.e. a mean of 44.7% from nominal) for endpoint derivation can be considered as conservative. Based on mean measured concentrations the 96-h E_rC_{50} was > 0.07 mg a.s./L and the E_bC_{50} was 0.007 mg a.s./L.

Because of the slight deviation in CV and the fact that chemical analyses were only performed on day 0 and 5, the study is considered as reliable with restrictions. It is relevant for classification purposes.

11.5.3.5 Toxicity to algae – *Ankistrodesmus bibraianus*

Author:	Backfisch, K. and Englert, D.
Title:	Effect of BAS 500 F (Pyraclostrobin, Reg.No. 304428) on the growth of the Green Alga *Ankistrodesmus bibraianus*
Date:	30.11.2018
Doc ID:	BASF DocID 2018/1099618, BASF Study Number 865309
Guidelines:	OECD 201 (2011)
GLP:	Yes
Validity:	Acceptable
Previous evaluation:	Submitted for the purpose of renewal

Material and Methods

Test item:	Pyraclostrobin (BAS 500 F; Reg. No.: 304428), batch no. L81-2, purity: 99.9 %
Test species:	Green alga, *Ankistrodesmus bibraianus,* strain 278/1; in-house cultures, originally obtained from "culture collection of algae and protozoa" (Scottish Marine Institute, Scotland, UK).
Test design:	Static system; test duration 72 hours; 5 test concentrations plus a dilution water and a solvent control; 5 replicates per treatment and dilution water control and 10 replicates for solvent control; daily assessment of growth.
Endpoints:	EC_x values with respect to growth rate and yield after exposure over 72 hours.
Test concentrations:	Control, solvent control (acetone), 0.0100, 0.0316, 0.100, 0.316 and 1.00 mg a.s./L (nominal).
Test conditions:	100 mL Erlenmeyer dimple flasks; test volume 60 mL; test medium: nutrient solution according to OECD TG 201 (2011); pH 8.1 at test initiation and pH 7.76 - 7.86 at test termination; temperature: 22 ±1 °C; initial cell densities: 1 x 10^4 cells/mL; continuous light at about 8000 lux; constant shaking (130 rpm).
Analytics:	Analytical verification of the test item was conducted using a LC-MS-method with MS-detection
Statistics:	Descriptive statistics; Student's t-test to compare control and solvent control; non-linear regression after a 3-parameter normal sigmoid model fit for determination of EC_x values; Shapiro-Wilks' Test and Levene's Test to check for normality and homogeneity of variance ($\alpha = 0.05$); multiple sequentially rejective Welch-t-test after Bonferroni-Holm ($\alpha = 0.05$) for determination of NOEC values with regard to growth and yield, respectively.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean measured values ranged from 89.7 to 97.9 % of nominal at test initiation and from 80.0 to 92.6% of nominal at the end of the test (Table 178). The biological results are based on nominal test concentrations.

Table 178: Analytical results at test start and after 72-hours

Nominal concentration (mg/L)	**0 hours**		**72 hours**	
	Measured concentration (mg/L)	**Recovery (%)**	**Measured concentration (mg/L)**	**Recovery (%)**
Control	< LOQ	-	< LOQ	-
Solvent control	< LOQ	-	< LOQ	-
0.010	0.00979	97.9	0.00898	89.8
0.0316	0.0283	89.7	0.0253	80.0
0.10	0.0963	96.3	0.0909	90.9
0.316	0.2924	92.5	0.290	91.8
1.00	0.941	94.1	0.926	92.6

LOQ = limit of quantification (0.0001 mg/L)

Biological results: No morphological changes on algal cells were observed in all test item concentrations and the control groups. After 72 h of exposure, statistically significant effects compared to the solvent control were detected at the four highest test item concentrations for growth rate and yield (Welch t-test after Bonferroni-Holm, $p < 0.05$). The effects on algal growth are summarized in Table 179.

Table 179: Effect of pyraclostrobin on the growth of the diatom *Ankistrodesmus bibraianus*

Concentration [mg a.s./L] (nominal)	**Solvent control**	**0.0100**	**0.0316**	**0.100**	**0.316**	**1.00**
Inhibition in 72 h (growth rate) [%] #	-	0.4	7.9 1)	25.7 1)	61.2 1)	89.3 1)
Inhibition in 72 h (yield) [%] #	-	1.4	24.0 1)	59.9 1)	90.2 1)	98.4 1)
Endpoints [mg pyraclostrobin/L] (nominal)						
E_rC_{10} (72 h)	0.0445 (95% confidence limits: 0.0381 – 0.0520)					
E_rC_{20} (72 h)	0.0771 (95% confidence limits: 0.0663 – 0.0897)					
E_rC_{50} (72 h)	0.221 (95% confidence limits: 0.184 – 0.265)					
E_yC_{10} (72 h)	0.0169 (95% confidence limits: 0.0143 – 0.0200)					
E_yC_{20} (72 h)	0.0279 (95% confidence limits: 0.0237 – 0.0329)					
E_yC_{50} (72 h)	0.0732 (95% confidence limits: 0.0599 – 0.0892)					

Growth inhibition relative to solvent control is given.

1) Statistically significant differences compared to the control (Welch t-test, one-sided smaller, after Bonferroni-Holm, $\alpha = 0.05$).

Validity criteria according to OECD TG 201 (2011)	**Obtained in this study**
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92/day (for species in Annex 2 of OECD TG 201)	29-fold increase
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%.	6.5%
The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata*	1.1%

and *Desmodesmus subspicatus*. For other less frequently tested species, the value should not exceed 10%.	

Conclusions

The E_rC_{50} (72 h) for pyraclostrobin was determined to be 0.221 mg a.s./L, ErC10 was 0.0445 mg/a.s./L, based on nominal concentrations. Based on OECD 201 (2011) validity criteria the study can be considered as valid.

The results are considered reliable. The study is relevant for classification purposes.

11.5.3.6 Toxicity to algae – *Navicula pelliculosa*

Author:	Eckenstein, H.
Title:	BAS 500 F (Pyraclostrobin) – Effect on *Navicula pelliculosa* in a 72-hour algal growth inhibition test
Date:	06.12.2018
Doc ID:	BASF DocID 2018/1194277, BASF Study Number 876249
Guidelines:	OECD 201 (2011)
GLP:	Yes
Validity:	Acceptable
Previous evaluation:	Submitted for the purpose of renewal

Material and Methods

Test item:	Pyraclostrobin (BAS 500 F; Reg. No.: 304428), batch no. L81-2,
Test species:	Fresh water diatom, *Navicula pelliculosa* (Hilse); Strain No. 1050-3 SAG; in-house culture, originally obtained from the collection of Algal Cultures (SAG, Institute for Plant Physiology, University of Göttingen, Germany).
Test design:	Static system; test duration 72 hours; 6 test concentrations plus a control, each with 4 replicates per treatment and control group; daily assessment of growth.
Endpoints:	EC_x values with respect to growth rate and yield based on cell density after exposure over 72 hours.
Test concentrations:	Control (dilution water), six test item dilutions of a saturated stock solution resulting in geometric mean measured concentrations of 0.0105, 0.0345, 0.104, 0.340, 1.09 and 1.35 mg a.s./L.
Test conditions:	125 mL Erlenmeyer glass flasks; test volume 50 mL; test medium: AAP medium, supplemented with Na_2SiO_3 x $9H_2O$ and Na_2SeO_3; pH 6.9 - 8.0; temperature: 23 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at 4520 - 4690 lux; constant shaking (125 rpm).
Analytics:	Analytical verification of test item concentrations was conducted using an LC-method with MS/MS-detection
Statistics:	Descriptive statistics; Probit Analysis using linear maximum likelihood regression (EC_x) and Williams' t-test and Welch's t test with Bonferroni-Holm-adjustment test procedure, where appropriated (one-sided smaller, $\alpha = 0.05$) (NOEC).

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The analytically determined concentrations of

pyraclostrobin at test termination ranged from 71 to 85% of the initial measured concentrations. The following biological results are based on geometric mean measured concentrations.

The results of the chemical analysis are shown in Table 180 (extracted from study report):

Table 180: Analytical results

Dilution°	Analytically Measured Concentration of BAS 500 F [mg/L]		Mean Measured Concentration of BAS 500 F (Geometric Mean) [mg/L]
	0 Hours	72 Hours	
1:320	0.0116	0.00945	0.0105
1:100	0.0385	0.0310	0.0345
1:32	0.124	0.0874	0.104
1:10	0.372	0.311	0.340
1:3.2	1.176	1.003	1.09
1:2.5	1.501	1.216	1.35

°: Dilutions of an equilibrated test item solution with a loading rate of 10 mg/L

Biological results: No morphological effects on algal cells were observed in the 1.09 mg/L treatment compared to the control. After 72 h of exposure, statistically significant effects compared to the control were detected at test item concentrations ≥ 0.104 mg a.s./L for growth rate (Williams t- test, $p < 0.05$) and yield (Welch's t-test, one-sided, $p < 0.05$). At the test item concentrations of 0.104 and 0.340 mg/L the mean inhibition of growth rate compared to the control was 6.1 and 3.7 %, respectively. This statistically significant finding was not considered as a biologically relevant toxic effect, since the mean inhibitions compared to the control were below 10% and showed no clear dose response effect at these concentrations. The effects on algal growth are summarized in Table 181.

Table 181: Effect of pyraclostrobin on the growth of the diatom *Navicula pelliculosa*

Concentration [mg a.s./L] (geometric mean measured)	**Control**	**0.0105**	**0.0345**	**0.104**	**0.340**	**1.09**	**1.35**
Inhibition in 72 h (growth rate) [%]	-	2.9	0.8	6.1 [1) 3)]	3.7 [1) 3)]	23.6 [1)]	49.7 [1)]
Inhibition in 72 h (yield) [%]	-	9.9	2.4	20.3 [2)]	13.0 [2)]	59.6 [2)]	86.0 [2)]
Endpoints [mg pyraclostrobin/L] (geometric mean measured)							
E_rC_{10} (72 h)	0.675 (95% confidence limits: 0.593 - 0.741)						
E_rC_{20} (72 h)	0.883 (95% confidence limits: 0.818 - 0.936)						
E_rC_{50} (72 h)	> 1.35 (extrapolated: 1.48 (95% confidence limits: 1.41 - 1.57))						
E_yC_{10} (72 h)	0.313 (95% confidence limits: 0.220 - 0.393)						
E_yC_{20} (72 h)	0.429 (95% confidence limits: 0.328 - 0.513)						
E_yC_{50} (72 h)	0.784 (95% confidence limits: 0.687 - 0.874)						

1) Statistically significant differences compared to the control (William's t-test, one-sided smaller, $\alpha = 0.05$).
2) Statistically significant differences compared to the control (Welch's t-test, one-sided smaller, $\alpha = 0.05$).
3) Statistically significant differences were assumed not to be biologically relevant toxic effects, since the mean inhibitions compared to the control were below 10% and showed no clear dose response effect.

Validity criteria according to OECD TG 201 (2011)	Obtained in this study
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92/day (for species in Annex 2 of OECD TG 201)	40-fold increase (72 h)
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%.	33 % (72 h)
The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with Pseudokirchneriella subcapitata and Desmodesmus subspicatus. For other less frequently tested species, the value should not exceed 10%.	1.6 % (72 h)

Conclusions

In a 72-hour static alga test with *Navicula pelliculosa*, the E_rC_{50} (72 h) for pyraclostrobin was determined to be > 1.35 mg a.s./L based on geometric mean measured concentrations.

Based on OECD 201 (2011) validity criteria the study can be considered as valid and reliable. It is relevant for classification purposes.

11.5.3.7 Toxicity to aquatic plants – *Lemna gibba*

Author:	Boeri R.L. et al. Anonymous
Title:	Growth and reproduction toxicity test with BAS 500F and the duckweed, *Lemna gibba* G3 Addendum study BASF DocID: 2000/5037
Date:	23.02.2000 07.06.2019
Doc ID:	1324-BA; BASF Reg. Doc. Number 2000/5037 BASF DocID 2019/2036269
Guidelines:	EPA 123-2, EPA 850.4400
GLP:	Yes (certified by United States Environmental Protection Agency)
Validity:	Acceptable (based on US EPA Guideline and OECD 221)
Previous evaluation:	Submitted for the purpose of renewal

Material and Methods

Test item:	BAS 500 F (batch no. 27882/191/C); purity: 97.09 %
Test species:	Duckweed (*Lemna gibba* G3)
Test design:	Static system; test duration 14 days; 5 test item concentrations plus a control and a solvent control, 3 replicates for each test item concentration, the control and the solvent control; 3 plants with 3 - 4 fronds, total number of fronds at test initiation: 9 - 11 per replicate; assessment of growth and other effects on days 1, 4, 6, 8, 11 and 14.
Endpoints:	EC_{25}, EC_{50} based on biomass and based on number of fronds, observation of chlorotic fronds
Test concentrations:	Control, solvent control (0.1 mL dimethylformamide/L), 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.s./L (nominal), corresponding to geometric mean measured concentrations of 0.068, 0.119, 0.242, 0.386 and 1.077 mg a.s./L.

Test conditions:	500 mL glass flasks, test volume: 200 mL, M-Hoagland's media without sucrose or EDTA, pH 4.9 - 5.1 at test initiation and pH 5.5 - 5.7 at test termination; temperature: 24.7°C - 25.5°C, continuous light, light intensity: about 490 foot candles.
Analytics:	HPLC
Statistics:	Descriptive statistics, t-test ($\alpha = 0.05$) for comparison of frond no. and dry weight in the control and solvent control, weighted least squares non-linear regression for determination of EC_x values based on frond no. and dry weight, Bonferroni's test for determination of the NOEC value ($\alpha = 0.05$).

Results and Discussion

Analytical measurements: Test item concentrations were analyzed in each concentration at the beginning and at the end of the test. The analyzed contents of pyraclostrobin ranged from 81 to 92% of nominal at test initiation and from 17% to 34% of nominal at test termination. Hence the biological results were based on geometric mean measured concentrations. The results of the chemical analysis are shown in Table 182 (extracted from study report):

Table 182: Analytical results

Nominal Concentration of BAS 500 F (mg/L)	Measured Concentration of BAS 500 F (mg/L)			
	Day 0	% Recovery	Day 14	% Recovery
Test Media				
0 (control)	ND[1]	---	ND	---
0 (solvent control)	ND	---	ND	---
0.13	0.120	92	0.0383	29
0.25	0.202	81	0.0700	28
0.50	0.422	84	0.139	28
1.0	0.896	90	0.166	17
2.0	1.72	86	0.674	34
Blank				
0	ND	---	ND	---
Matrix Spike[2]				
0.50	--- ---	---	0.434 0.366	87 73
Laboratory Control Spike[2]				
0.50	0.455	91	0.389	78

[1] ND = none detected at or above the limit of quantitation of 0.00050 mg/L.
[2] Matrix spike samples and laboratory control samples were prepared with the test substance used to formulate the test concentrations (purity = 97.09%).

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. The duckweed population in the control vessels showed sufficient growth, increasing from an average of 11 fronds per vessel to an average of 198 fronds per vessel, corresponding to an 18 x multiplication. At the end of the test, chlorotic fronds were observed in the control, the solvent control and in all test item concentrations tested. Statistically significant effects on the number of normal, non-chlorotic fronds and the plant biomass compared to the pooled controls were observed at the highest tested concentration of 1.077 mg a.s./L (Bonferroni's test; $\alpha = 0.05$). Effects on biomass development are summarized in Table 183.

Table 183: Effects of pyraclostrobin on the biomass development of *Lemna gibba*

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.13**	**0.25**	**0.50**	**1.0**	**2.0**
Concentration [mg a.s./L] (geometric mean measured)	–	–	0.068	0.119	0.242	0.386	1.077
Inhibition based on growth rate [% of pooled control] *	--	--	-2.05	2.03	1.82	1.45	15.9
Inhibition based on yield [% of pooled control] *	--	--	-6.74	11.1	12.3	3.7	43.2
Inhibition based on dry weight [% of pooled control] *	--	--	-6.1	5.8	9.6	1.8	34.5
Endpoints [mg pyraclostrobin/L] (geometric mean measured)							
E_rC_{50} (14 d) based on frond no.	> 1.077						
E_rC_{20} (14 d) based on frond no.	> 1.077						
E_rC_{10} (14 d) based on frond no.	0.820 (95% CI: 0.473 to 1.420)						
E_yC_{50} (14 d) based on frond no.	> 1.077						
E_yC_{20} (14 d) based on frond no.	0.648 mg/L (95% CI: 0.021 to 2.073)						
E_yC_{10} (14 d) based on frond no.	0.454 mg/L (95% CI: 0.141 to 1.458)						
E_bC_{50} (14 d) based on dry weight	> 1.077						
E_bC_{20} (14 d) based on dry weight	0.751 mg/L (95% CI: 0.2064 to 2.813)						
E_bC_{10} (14 d) based on dry weight	0.513 mg/L (95% CI: 0.146 to 1.797)						

* negative values indicate stimulated growth

Validity criteria according to OECD TG 221 (2006)	**Obtained in this study**
...the doubling time of frond number in the control must be less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275/day	> 7-fold increase (8 d) *

* Since no assessment of frond no. was done after 7 days, frond no. data after 8 day are considered for validity check.

Conclusions

In a 14-day aquatic-plant test with *Lemna gibba*, the E_rC_{50} and the E_yC_{50} of pyraclostrobin based on frond number and the E_bC_{50} based on dry weight were determined to be all > 1.077 mg a.s./L, based on geometric mean measured concentrations. It should be noted, that this study was conducted in accordance with an US EPA Guideline. Thus, the study duration is double of the duration recommended by current OECD TG 221 (2006). All validity criteria were met.

The study is valid and considered reliable. It is relevant for classification purposes.

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

No data available.

11.6 Long-term aquatic hazard

All the information on chronic toxicity are taken from the RAR and list of endpoints for pyraclostrobin, January 2020.

Table 184: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
OECD 204	*Oncorhynchus mykiss*	Pyraclostrobin (99 %)	$NOEC_{mortality}$ (28 d) = 0.0031 mg a.s./L (mean measured) – flow-through	Reliability: additional information* No reliable EC_{10}/EC_{20} could be derived Also fulfills validity criteria of OECD TG 215	42F0494/965177: 1999
OECD 210	*Oncorhynchus mykiss*	Pyraclostrobin (99 %)	$NOEC_{survival}$ (98 d) = 0.00235 mg a.s./L (mean measured) – flow-through	Key study Reliability: 1 No reliable EC_{10}/EC_{20} could be derived	52F0494/965141: 1999
EPA 72-4(a), EPA 850.1400	*Pimephales promelas*	Pyraclostrobin (97.1 %)	NOEC (36 d) = 0.00414 mg a.s./L (mean measured) – flow-through	Reliability: 1 No reliable EC_{10}/EC_{20} could be derived	1948-BA: 2000
EPA 72-4(a), EPA 850.1400	*Cyprinodon variegatus*	Pyraclostrobin (93.5 %)	NOEC (36 d) = 0.0108 mg a.s./L (mean measured) – flow-through	Reliability: 1 No reliable EC_{10}/EC_{20} could be derived	2126-BA: 2001
OECD 202 Part II (21 day reproduction test considering draft revision from January 1996)	*Daphnia magna*	Pyraclostrobin (97.1 %)	NOEC (21 d) = 0.004 mg a.s./L (nominal) – semistatic system	Reliability: 1 No reliable EC_{10}/EC_{20} could be derived	35811: Dohmen, 1999

Method	Species	Test material	Results[1]	Remarks	Reference
EPA, FIFRA 72-4 (b)	*Americamysis bahia*	Pyraclostrobin (99.7 %)	NOEC (28 d) = 0.0005 mg a.s./L (mean measured) – flow-through	Reliability: 3 Study not valid	7473-BA: Ward, 2004
EPA 850.1000, EPA 850.1350, EPA 72-4	*Americamysis bahia*	Pyraclostrobin (99 %)	$NOEC_{weight}$ (31 d) = 0.000365 mg a.s./L (mean measured) – flow-through	Key study Reliability: 1 No reliable EC_{10}/EC_{20} could be derived	68248: Dinehart, 2013 and study addendum 2019/2036254: Unknown, 2019
BBA-guideline proposal: 'Effects of plant protection products on the development of sediment dwelling larvae of Chironomus riparius in a water-sediment system.", Mitteilungen aus der Biol. Bundesanstalt, Heft 315, Blackwell Berlin, 1995, pp 70-84.	*Chironomus riparius*	Pyraclostrobin (97.1 %) + 14C radiolabelled pyraclostrobin (98 %)	NOEC (28 d, emergence) = 0.08 mg a.s./L (nominal water concentration) EC_{10} (28 d, emergence) = 0.129 mg a.s./L (nominal water concentration) – static system	Reliability: 3 Clear shift to sediment. It is not acceptable to base results on nominal water concentrations. Calculation of mean measured concentrations in sediment not possible.	35966: Dohmen, 2000
OECD 218 (2004)	*Chironomus riparius*	Pyraclostrobin (100 %)	NOEC (28 d, emergence) = 1.37 mg a.s./ kg dw (mean measured) – static system	Reliability: 2 Concentrations in sediment were only verified for two treatments.	74981250: Kuhl and Wydra, 2013
OECD 201	*Pseudokirchneriella subcapitata*	Pyraclostrobin (97.1 %)	E_rC_{10} (96 h) = 0.078 mg a.s./L E_rC_{10} (72 h) = 0.071 mg a.s./L (mean measured) – static exposure	Reliability: 1	35803: Dohmen, 1999 2009/1037148: Hoffmann, 2009 – additional calculation

Method	Species	Test material	Results[1]	Remarks	Reference
EPA 123-2, EPA 850.5400	*Navicula pelliculosa*	Pyraclostrobin (97.1 %)	NOE_rC (120 h) = 0.00118 mg a.s./L (initial measured) – static system	Reliability: 2	1321-BA: Boeri, 2000
EPA 123-2, EPA 850.5400	*Anabaena flos-aquae*	Pyraclostrobin (97.1 %)	NOE_rC (120 h) > 1.78 mg a.s./L (mean measured) – static system	Reliability: 3 Validity criteria not met	1222-BA: Boeri, 2000
EPA 123-2, EPA 850.5400	*Skeletonema costatum*	Pyraclostrobin (97.1%)	NOE_rC (96 h) = 0.0194 mg a.s./L (mean measured) – static system	Reliability: 2	1223-BA: Boeri, 2000
OECD 201 (2011)	*Ankistrodesmus bibraianus*	Pyraclostrobin (99.9 %)	E_rC_{10} (72 h) = 0.0445 mg a.s./L (nominal) – static system	Reliability: 1	2018/1099618: Backfisch and Englert, 2018
OECD 201 (2011)	*Navicula pelliculosa*	Pyraclostrobin (99.9 %)	E_rC_{10} (72 h) = 0.675 mg a.s./L (mean measured) – static system	Reliability: 1	2018/1194277: Eckenstein, 2018
EPA 123-2, EPA 850.4400 OECD 221	*Lemna gibba*	Pyraclostrobin (97.1 %)	E_rC_{10} (14 d) = 0.82 mg a.s./L (mean measured) – static system	Reliability: 1	1324-BA: Boeri, 2000 Combined with addendum study 2019/2036269: Unknown, 2019

[1] Indicate if the results are based on the measured or on the nominal concentration

* Tests performed according to OECD 204 or similar guidelines are not considered suitable long-term tests (Guidance on Information Requirements and Chemical Safety Assessment - Chapter R.7b: Endpoint specific guidance Version 3.0, February 2016). However, the validity criteria according to OECD 215 were also met, and sublethal endpoints recorded. The test is therefore considered as additional information for the assessment of long-term aquatic hazard.

11.6.1 Chronic toxicity to fish

11.6.1.1 Chronic toxicity to fish – *Oncorhynchus mykiss*

Author:	Anonymous
Title:	BAS 500 F - Sublethal toxic effects on the rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792) in a flow-through system (28 days)
Date:	10.09.1999
Doc ID:	42F0494/965177; BASF DocID 1999/11249
Guidelines:	OECD 204
GLP:	Yes
Validity:	Acceptable
Previous evaluation:	In initial DAR (2001)

Material and Methods

Test item:	BAS 500 F (substance number 96/494-3; batch no. CP 029 053); purity: 99.0 %
Test species:	Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792), mean body length 3.9 (3.5 – 4.3) cm, mean body weight 0.6 (0.5 – 0.65) g.
Test design:	Flow-through system (28 days), flow rate of the test solution 10 l/h/aquarium; 20 fish per aquarium. Four test item concentrations and a negative (water) control; one replicate each; daily assessment of mortality and symptoms of toxicity after start of exposure.
Endpoints:	NOEC, mortality and sub-lethal effects
Test concentrations:	Control, 0.00215, 0.00464, 0.01, 0.0215 mg as/L (nominal).
Test conditions:	Glass aquaria (60 x 35 x 40 cm), test volume: 60 L water; temperature: 15 +/- 1 °C; pH 8.1 – 8.3; dissolved oxygen concentration 8 - 10 mg/L; photoperiod: 16 h light : 8 h dark; no feeding.
Analytics:	HPLC with UV-detection.
Statistics:	Descriptive statistics; one-way analysis (ANOVA) followed by a Dunnett´s test for determination of the NOEC based on body weight and length data.

Results and Discussion

Analytical measurements: Analytical verification of test item concentration was conducted. The analyzed contents ranged from 64% to 101% of nominal, i.e. minimum and maximum of all samplings over the test duration (n = 5). For the concentration presenting the NOEC as mean of all samplings 66.1% of nominal were measured. The results of the analytically detected concentrations are shown in Table 185.

Table 185: Analytical results

Nominal concentrations (mg/L)	Number analytical determinations	Recovery based on mean of determinations (%)
Control	5	n.d.
0.00215	5	79.0
0.00464	5	66.1
0.010	5	79.5
0.0215	1	78.1

n.d. = not detectable

Biological results: Compound-related mortality occurred in the two highest test concentrations 0.01 and 0.0215 mg as/L. Compound-related toxic signs were observed only in the second highest concentration starting on day 1 in the form of reduced or no food uptake, convulsions and narcotic-like state. The one surviving fish showed sporadically apathy, reduced or no food uptake and swimming near the bottom. At test end no toxic signs were observed. The mean body weight and length at the end of the study was not statistically significantly different from the control group (Dunnett´s test). The results are summarized in Table 186.

Table 186: Chronic toxicity (28 d) of pyraclostrobin on rainbow trout (*Oncorhynchus mykiss)*

Concentration [mg a.s./L] (nominal)	Control	0.00215	0.00464	0.0100	0.0215
Mortality [%] (28 d)	0	0	0	95	100
Symptoms (28 d)	none	none	none	none	n.d.
Mean weight [g] (28 d)	1.90	1.84	1.86	1.30	n.d.

Concentration [mg a.s./L] (nominal)	**Control**	**0.00215**	**0.00464**	**0.0100**	**0.0215**
Mean length [cm] (28 d)	5.65	5.56	5.56	5.10	n.d.
Endpoints [mg pyraclostrobin/L]					
	based on nominal		**based on mean measured ***		
NOEC (28 d)	0.00464		0.0031		

n.d. = not determined; all fish dead
* Considering the mean measured recovery rate of 66.1% of nominal in the 0.00464 mg/L treatment (nominal)

Validity criteria according to OECD TG 215 (2000)	**Obtained in this study**
The mortality in the control(s) should not exceed 10 per cent at the end of the test	0%
Mean weight (in controls) of fish increased by 50% of the initial weight over 28 days	> 300% increase
The dissolved oxygen concentration must have been at least 60 per cent of the air saturation value throughout the test.	> 60 (7.8 – 10.1 mg/L)
Water temperature must not differ by more than ±1°C between test chambers and any one time during the test it should be maintained within a range of 2°C (12.5 – 16.0 °C for rainbow trout))	15 ±1°C (range: 14 – 16 °C)

Conclusions

In a flow-through chronic toxicity study with the rainbow trout the NOEC (28 d) of pyraclostrobin was 0.00464 mg a.s./L based on nominal concentrations. Considering that the analytical measurements showed only 66.1% of nominal for this concentration the NOEC based on the measured concentration was 0.0031 mg a.s./L.

Due to the steep dose response curve in this study and the low number of tested concentrations, no reliable EC_{10} and EC_{20} value could be derived from this study. Overall, effects ≥ 10% only occurred at test item concentrations greater than the NOEC. Thus, the NOEC is the more conservative endpoint.

The OECD TG 204 is no longer supported by EFSA and no valid guideline currently exists for this type of study. However, since all validity criteria according to current OECD TG 215 (fish juvenile growth test) are met, this study is considered valid.

The study is considered reliable. It is considerd as additional information for classification purposes.

11.6.1.2 Chronic toxicity to fish – *Oncorhynchus mykiss*

Author:	Anonymous
Title:	BAS 500 F - Early life-stage toxicity test on the rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792)
Date:	24.09.1999
Doc ID:	52F0494/965141; BASF DocID 1999/11343
Guidelines:	OECD 210
GLP:	Yes
Validity:	Acceptable
Previous evaluation:	In initial DAR (2001)

Material and Methods

Test item:	BAS 500 F (substance number 96/494-3; batch no. CP 029 053); purity: 99.0 %
Test species:	Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792), embryos (appr. 90 – 120 min after fertilization)
Test design:	Flow-through system, study was terminated on day 98, 60 days after completion of hatch (day 38); 4 replicates of 25 embryos per test vessel and per concentration; mortality was determined at least on workdays, time to hatch and swim up were determined, toxic signs and abnormalities were determined at least workdays, body weights and lengths were determined at the end of the study
Endpoints:	NOEC, mortality and sub-lethal effects
Test concentrations:	Control, 0.0001, 0.000316, 0.001, 0.00316, 0.01 mg as/L (nominal)
Test conditions:	Aquaria (29 x 21 x 22 cm) with a test volume of 9 L water; temperature: 10 +/- 1 °C; pH 7.3 – 8.2; dissolved oxygen concentration 10.5 – 11.5 mg/L; until swim-up aquaria were kept in the dark after swim-up dim light with a cycle of 16 hours light and 8 hours dark; flow rate: 10 L/hour/test group
Analytics:	RP-HPLC
Statistics:	Descriptive statistics; DUNETTs test (two-sided) for body weight and length, log-rank test (two-sided) for survival

Results and Discussion

Analytical measurements: Analytical verification of test item concentration was conducted. The analyzed contents ranged from 60.7% to 80.6% of nominal. The analytical results are presented in Table 187.

Table 187: Analytical results

Nominal concentration (mg/L)	Analytically determined concentration ± standard deviation (mg/L)	Mean recovery (%)
0.0001	0.0000806 ± 0.000016	80.6
0.000316	0.000208 ± 0.000035	65.8
0.001	0.000607 ± 0.000108	60.7
0.00316	0.002349 ± 0.000368	74.3
0.01	0.006419 ± 0.000535	64.2

Biological results: In the highest test concentration 0.01 mg as/L all fish died until day 44. This was considered to be a clear substance related effect. No significant deviation in survival from the control group was seen in the dose groups 0.001 and 0.00316 mg as/L until the end of the study. Therefore, the significant decrease in survival for the time period 0-56 days observed in the concentration group 0.000316 mg as/L was considered to be not test com-pound related.

The larvae of the highest concentration group (0.01 mg as/L) showed apathy and partly a narcotic state and a distended yolk-sac for a period of 6 days after the end of hatch until all larvae of this group had died. In the other concentration groups effects were limited to single individuals and are judged not to be compound-related. No external abnormalities were observed in the surviving animals at the end of the study.

Due to an infection of the control group, a second control group of another study conducted parallel with eggs from the same hatch was used additionally.

Table 188: Chronic toxicity of pyraclostrobin to rainbow trout (*Oncorhynchus mykiss*) in a fish early life stage test (98 d)

Concentration [mg a.s./L] (nominal)	**Control**	**0.00010**	**0.000316**	**0.0010**	**0.00316**	**0.010**
Concentration [mg a.s./L] (mean measured)	**--**	**0.0000806**	**0.000208**	**0.000607**	**0.00235**	**0.00642**
Start of hatch [day]	34	34	34	32	33	32
End of hatch [day]	38	38	38	37	38	38
Time to swim-up [day]	56	56	54	54	56	-
Survival of larvae at day 56 [%]	74	69	62	72	67	0 *
Survival of young fish at day 98 related to day 56 survivors [%]	91.9	98.6	96.8	95.8	94.0	-
Young fish mean survival rate (0-98 d) [%]	68	68	60	68.7	63	-
Mean wet weight [% of 1st control][1)]	100	277.3	249.8	264.8	254.3	-
Mean wet weight [% of 2nd control][2)]	100	94.5	85.1	90.2	86.7	n.d.
Mean body length [% of control][1)]	100	130.9	129.2	129.0	124.6	n.d.
Mean body length [% of control][2)]	100	100.4	99.1	98.9	95.5	n.d.
Symptoms [#]	none	A+	A+	none	A+	n.d.
Endpoint [mg pyraclostrobin/L] (mean measured)						
$NOEC_{overall}$ (98 d)	0.00235					

[#] Symptoms: A = apathy; [+] = One specimen only

* Statistically significant adverse test-item related effect (log-rank test (two-sided) for survival; $p < 0.01$ for survival data).

[1)] The control was adversely affected probably by an infection. Therefore, the food uptake was reduced in the control group for the last three weeks of the exposure resulting in a significant decrease of the body weight and length in comparison to the dose groups.

[2)] The body weight and length of a range finding test conducted in parallel to this study were used additionally for the evaluation of effects

Validity criteria according to OECD TG 210 (2013)	**Obtained in this study**
The dissolved oxygen concentration should be > 60% of the air saturation value throughout the test.	> 60% (10.5 – 11.5 mg/L)
The water temperature should not differ by more than ±1.5°C between test chambers or between successive days at any time during the test and should be within the temperature ranges specified for the test species (10 °C ±1.5 °C).	Temp. range: 10 – 11 °C Differences between test chambers / between successive days < 1.5 °C
The analytical measure of the test concentrations is compulsory. When the measured concentrations do not remain within 80-120% of the nominal concentration, the effect concentrations should be determined and expressed relative to the arithmetic mean concentration for flow-through tests.	60.7 to 80.6% of nominal throughout the test; therefore, results are based on mean measured concentrations
Overall survival of fertilized eggs and post-hatch success in the controls and, where relevant, in the solvent controls should be greater than or equal to the limits defined (for rainbow trout: 75% hatching success, 75% post-hatch success).	96.2% hatching success and 91.9% post-hatch success in control

Survival rate in concurrent viability control (mean of 200 embryos) after 14 d was 78%

Conclusions

In an early life stage study with rainbow trout (*Oncorhynchus mykiss*) the overall NOEC (98 d) for pyraclostrobin was determined to be 0.00235 mg a.s./L based on mean measured concentrations.

Due to the steep dose-response curve in this study no reliable EC_{10} and EC_{20} value could be derived from this study. Overall, effects ≥ 10% (compared to control) only occurred at test item concentrations greater than the NOEC. Thus, the NOEC is the more conservative endpoint.

It should be noted that due to an infection of the control group, a second control group of another study conducted parallel with eggs from the same hatch was used additionally for this study (see biological results). As it was a control group run in parallel with the same conditions and performed with eggs from the same hatch there is no impact on the study outcome.

The study is valid and considered reliable. It is relevant for classification purposes.

11.6.1.3 Chronic toxicity to fish – *Pimephales promelas*

Author:	Anonymous
Title:	Early life stage toxicity of BAS 500 F to the fathead minnow, *Pimephales promelas*
Date:	29.03.2000
Doc ID:	1948-BA; BASF Reg. Doc. Number 2000/5053, BASF Study Number 63932
Guidelines:	EPA 72-4(a), EPA 850.1400
GLP:	Yes
Validity:	Acceptable (some minor deviations not influencing the study results and acceptability)
Previous evaluation:	Submitted for the purpose of renewal

Material and Methods

Test item:	Pyraclostrobin (BAS 500, lot no. 27882/191/C); purity: 97.09 %
Test species:	Fathead minnow (*Pimephales promelas*), eggs less than 24 hours at test initiation, source: "Aquatic BioSystems, Inc.", Fort Collins, Colorado, USA.
Test design:	Flow-through system (36 d); 5 test item concentrations plus a dilution water control and a solvent control; 4 replicate test chambers per treatment with 20 fertilized eggs in each; a proportional diluter system was used for intermittent introduction of the solutions to the test chambers. During the embryo stage, the developing embryos were incubated in glass cups. At the end of hatch (day 4), fish were released into the test chamber and randomly thinned to 10 fish per vessel. Daily assessment of hatch, survival, signs of toxicity and abnormal behavior. At test termination surviving animals were sacrificed and measured for length and weight.
Endpoints:	NOEC values based on hatchability, survival, toxic signs and growth rates.
Test concentrations:	Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.0014, 0.0026, 0.0049, 0.010 and 0.020 mg a.s./L (nominal), corresponding to mean measured concentrations of 0.000944, 0.00218, 0.00414, 0.00837 and 0.0161 mg a.s./L.
Test conditions:	Test vessels: 9 L glass aquaria (approx.15 x 30 x 20 cm) with a test volume of approx. 8.0 L; 4 replicate test chambers; glass incubation cups (used during embryo stage) closed on one end with Nitex® screen; two incubation cups per test chamber; dilution water: filtered deionized water sterilized with UV and aerated; temperature: 23.0°C - 26.4°C; pH 7.4 - 7.8; oxygen content: 8.0 mg/L - 9.4 mg/L; total hardness: 40 - 44 mg CaCO3/L;

	conductivity: 130 - 180 µmhos/cm; light intensity: approx. 45 foot candles; photoperiod: 16 hours light : 8 hours dark; flow rate: approx. 6.6 volume additions per 24 hours per vessel; feeding: fish were fed 2-3 times daily ad libitum freshly hatched Artemia salina nauplii from day 5 onwards until 1 day before study termination; no aeration.
Analytics:	HPLC-method with UV-detection
Statistics:	Descriptive statistics; t-test for comparison of the dilution water control and solvent control data; ANOVA followed by Bonferroni's test or William's test to calculate NOEC values.

Results and Discussion

Analytical measurements: Analytical verification of the test item concentrations was conducted in all concentrations at test initiation, at regular intervals during the study and test end, except for the highest test item concentration, where analytical measurements were only conducted on days 0 and 7. Recoveries of pyraclostrobin ranged from 74.5 to 90.0% of nominal concentrations in all treatments at test initiation and from 80.9 to 88.5% of nominal in the 0.0026, 0.0049 and 0.010 mg/L (nominal) treatments at test termination. Measured contents in samples from the lowest test item treatment were below the limit of quantification (LOQ = 0.50 µg/L) on days 35 and 36; this is believed to be due to an unobserved diluter malfunction on test days 35 and 36. All analysis at this concentration prior to that time resulted in recoveries ranging from 75 to 84% of nominal. The following biological results are based on mean measured concentrations. The results of the chemical analysis are shown in Table 189.

Table 189: Analytical results

Nominal concentration (mg/L)	**Measured concentration (mg/L)**								**Percent of nominal**
	Day of exposure								
	0	**7**	**14**	**21**	**28**	**35**	**36**	**Mean**	
Control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	--	--
Solvent control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	--	--
0.0014	0.0016	0.0011	0.0011	0.0012	0.0011	n.d. [1]	n.d. [1]	0.0009 [1]	67
0.0026	0.0020	0.0022	0.0022	0.0022	0.0022	0.0022	0.0023	0.0022	84
0.0049	0.0044	0.0045	0.0039	0.0040	0.0038	0.0042	0.0041	0.0041	84
0.0100	0.0076	0.0092	0.0078	0.0087	0.0088	0.0084	0.0081	0.0084	84
0.0200	0.0149	0.0173	--	--	--	--	--	0.0161	81

n.d. = not detectable

[1] As each sample (alalyzed twice) resulted in n.d. concentrations, the LOQ (= 0.005 mg/L) was considered at day 35 and 36.

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. Egg hatch was complete on day 4 in the control and all test item treatments. Mean survival rates at hatch (day 4) and at test end (32 days post-hatch) were ≥ 90% in both the dilution water control and solvent control and at test item concentrations up to and including 0.00837 mg a.s./L, whereas all fish had died before hatch (day 4) in the highest tested concentration of 0.0161 mg a.s./L. No statistically significant effects on survival, time to hatch, time to first feeding, total length, wet weight or dry weight compared to the pooled control were observed at concentrations of up to and including 0.00414 mg a.s./L (Bonferroni's test / William's test). Sublethal effects, observed as fish exhibiting lethargy and/or a loss of equilibrium or change in coloration, were noted at 0.0161 mg a.s./L on day 3 (complete mortality occurred on day 4 at this concentration), at 0.00837 mg a.s./L on days 4 - 6, 8 - 12, and on day 34, and at 0.00414 mg a.s./L on day 4. These effects were not observed at any other time during the test. As the effect on live, normal fathead minnow at 0.00414 mg/L was apparent on only 4% of the fish on one day (day 4), and although statistically significant, the effect was not considered to be of biological relevance. The results

are summarized in Table 190.

Table 190: Chronic toxicity of pyraclostrobin to fathead minnow (*Pimephales promelas*) in an fish early life-stage test (36 d)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0014**	**0.0026**	**0.0049**	**0.010**	**0.020**
Concentration [mg a.s./L] (mean measured)	--	--	**0.000944**	**0.00218**	**0.00414**	**0.00837**	**0.0161**
Embryo survival at hatch on day 4 [%]	98	99	99	99	94	90	0
Survival of larvae 32 days post hatch [%]	100	98	95	95	100	90	0
Percent live normal at hatch on day 4 [%]	98	99	99	99	90[#]	80	0
Percent live normal 32 days post hatch [%]	100	98	95	95	100	90	0
Mean total length [mm]	23.3	23.0	23.3	22.8	23.0	22.3	n.d.
Mean wet weight [mg]	110.5	108.8	108.5	106.9	107.0	96.8	n.d.
Mean dry weight [mg]	23.6	23.0	23.2	22.7	23.3	22.4	n.d.
Endpoints [mg pyraclostrobin/L] (mean measured)							
$NOEC_{overall}$ (36 d)	0.00414						

n.d. = not determined; no fish survived at the concentration above 0.00837 mg a.s./L.

[#] Because the effect on live, normal fathead minnow at 0.00414 mg a.s/L was apparent on only 4% of the fish on one day (day 4), the effect was not considered to be of biological significance.

Validity criteria according to OECD TG 210 (2013)	**Obtained in this study**
The dissolved oxygen concentration should be >60% of the air saturation value throughout the test.	> 60% (8.0 – 9.4 mg/L)
The water temperature should not differ by more than ±1.5°C between test chambers or between successive days at any time during the test and should be within the temperature ranges specified for the test species (for fathead minnow: 25°C ±1.5°C).	Temp. range: 23.0 – 26.4°C Differences between test chambers / between successive days > 1.5°C
The analytical measure of the test concentrations is compulsory. When the measured concentrations do not remain within 80-120% of the nominal concentration, the effect concentrations should be determined and expressed relative to the arithmetic mean concentration for flow-through tests.	75 – 84% of nominal throughout the test; therefore, results are based on mean measured concentrations
Overall survival of fertilized eggs and post-hatch success in the controls and, where relevant, in the solvent controls should be greater than or equal to the limits defined in Annex 2 (for fathead minnow: 70% hatching success, 75% post-hatch success).	98% hatching success and 100% post-hatch success in control; 98% hatching success and 98% post-hatch success in solvent control

Failure in terms of meeting one validity criterion is marked in **bold.**

Conclusions

In an early life-stage study with fathead minnow (*Pimephales promelas*) the overall NOEC (36 d) for pyraclostrobin was determined to be 0.00414 mg a.s./L based on mean measured concentrations.

Due to the steep dose-response curve in this study no reliable EC_{10} and EC_{20} value could be derived from this study. Overall, effects ≥ 10% only occurred at test item concentrations greater than the NOEC. Thus, the NOEC is the more conservative endpoint.

Three out of the four validity criteria were met. The temperatures measured during the toxicity test were slightly below the recommended minimum temperature of 23.5°C (in the controls during week 2) and differences between test chambers or between successive days were > 1.5°C on several occasions. However, the deviations were only minor and were observed in all treatments during the whole study conduct. Overall, these deviations are of no relevance for the overall outcome of the study.

The study is considered valid and reliable. It is relevant for classification purposes.

11.6.1.4 Chronic toxicity to fish – *Cyprinodon variegatus*

Author:	Anonymous
Title:	Early life stage toxicity of BAS 500 F to the sheepshead minnow, *Cyprinodon variegatus*
Date:	25.05.2001
Doc ID:	2126-BA; BASF Reg. Doc. Number 2000/5247, BASF Study Number 64548
Guidelines:	EPA 72-4(a), EPA 850.1400
GLP:	Yes
Validity:	Acceptable (temperature was not in line with recent US EPA recommendation and OECD 210 for tested species, however there was no impact on the outcome of the study)
Previous evaluation:	Submitted for the purpose of renewal

Material and Methods

Test item:	BAS 500 F (Reg. No. 304 428, lot no. N68); purity: 93.5%
Test species:	Sheepshead minnow (*Cyprinodon variegatus*); eggs less than 24 hours before test initiation, source: "Aquatic BioSystems, Inc.", Fort Collins Colorado, USA
Test design:	Flow-through system (36 d); 5 test item concentrations plus a dilution water control and a solvent control; 4 replicate test chambers per treatment with 20 fertilized eggs in each; a proportional diluter system was used for intermittent introduction of the solutions to the test chambers. During the embryo stage, the developing embryos were incubated in glass cups. At the end of hatch (day 4 and 5), fish were released into the test chamber and randomly thinned to 10 fish per vessel. Daily assessment of hatch, swim-up, survival, signs of toxicity and abnormal behavior. On day 36 surviving animals were sacrificed and measured for length and weight.
Endpoints:	NOEC values based on hatch rate, post-hatch survival, sublethal effects, growth and time spans to hatch.
Test concentrations:	Control (dilution water), solvent control (0.1 mL dimethylformamide/L) and 0.0034, 0.0065, 0.013, 0.025 and 0.050 mg pyraclostrobin/L (nominal); corresponding to mean measured concentrations of 0.00291, 0.00557, 0.0108, 0.0240 and 0.0445 mg a.s./L.
Test conditions:	Test vessels: 9 L glass aquaria (15 x 30 x 20 cm) with a test volume of approx. 7.0 L; 4 replicate test chambers; glass incubation cups (used during embryo stage) with 8.5 cm diameter closed on one end with Nitex® screen; two incubation cups per test chamber; dilution water: filtered natural seawater diluted with deionized water,; water temperature: 29.0°C - 30.8°C; pH 7.5 - 8.1; oxygen content: 5.4 mg/L - 7.9 mg/L; salinity: 15 - 16 ppt; light intensity: approx. 42 foot candles; photoperiod: 16 hours light : 8 hours dark; flow rate: approx. 6.7 volume additions per 24 hours per vessel; feeding: fish were fed 2-3 times daily

	ad libitum freshly hatched Artemia salina nauplii from day 6 onwards until 1 day before study termination; no aeration.
Analytics:	HPLC-method with UV-detection
Statistics:	Descriptive statistics; t-test for comparison of the dilution water control and solvent data (α = 0.05); ANOVA followed by Bonferroni's test, William's test or Kruskal & Wallis's test to calculate NOEC values.

Results and Discussion

Analytical measurements: Analytical verification of the test item concentrations was conducted in all concentrations at test initiation, at regular intervals during the study and at test end, except for the highest test item concentration, where analytical measurements were only conducted on days 0 and 7. Recoveries of pyraclostrobin ranged from 75.3 to 103.1% of nominal concentrations at test initiation and from 79.4 to 84.0% of nominal at test termination. The biological results are based on mean measured concentrations. The results of the chemical analysis are shown in Table 191.

Table 191: Analytical results

Nominal concentration (mg/L)	**Measured concentration (mg/L)**								**Percent of nominal**
	Day of exposure								
	0	**7**	**14**	**21**	**28**	**35**	**36**	**Mean**	
Control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	--	--
Solvent control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	--	--
0.0034	0.0026	0.0032	0.0031	0.0030	0.0030	0.0029	0.0027	0.0029	86
0.0065	0.0067	0.0051	0.0052	0.0057	0.0055	0.0057	0.0052	0.0056	86
0.0130	0.0133	0.0094	0.0112	0.0104	0.0105	0.0105	0.0104	0.0108	83
0.0250	0.0256	0.0276	0.0243	0.0233	0.0235	0.0229	0.0210	0.0240	96
0.0500	0.0417	0.0473	--	--	--	--	--	0.0445	89

n.d. = not detectable

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; α = 0.05). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. Egg hatch was complete on day 4 in the control groups and the three lowest test item treatments. At 0.0240 mg a.s./L egg hatch was completed on day 5. In the highest test item concentration of 0.0445 mg a.s./L, all animals were dead before hatch (day 4). Mean survival rates at hatch (day 4) and at test end (32 days post-hatch) were ≥ 95 % in both the dilution water control and the solvent control and in test item concentrations of up to and including 0.0108 mg a.s./L. At 0.0240 mg a.s./L, mean survival was 29 % at hatch and 55% on day 32 post-hatch, respectively. No statistically significant effects on survival, time to hatch, time to first feeding, total length, wet weight or dry weight compared to the pooled control were observed at concentrations of up to and including 0.0108 mg a.s./L (Bonferroni's test / William's test / Kruskal & Wallis's test). Sublethal effects (i.e., lethargy and/or erratic swimming) were noted at 0.0108 mg a.s./L on day 4 (at hatch) and at 0.0240 mg a.s./L on days 4 and 5. These effects were not observed at any other time during the test. No other sublethal effects (other than delayed hatch) were observed at any test concentration at any time during the test. The results are summarized in Table 192.

Table 192: Chronic toxicity of pyraclostrobin to sheepshead minnow (*Cyprinodon variegatus*) in an fish early life stage test (36 d)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0034**	**0.0065**	**0.013**	**0.025**	**0.050**
Concentration [mg a.s./L] (mean measured)	--	--	**0.00291**	**0.00557**	**0.0108**	**0.0240**	**0.0445**
Embryo survival at hatch on day 4 [1] [%]	100	95	98	99	99	29	0
Survival of larvae 32 days post hatch [%]	95	100	95	95	98	55	0
Percent live normal at hatch on day 4 [%]	100	95	98	99	98	9	0
Percent live normal 32 days post hatch [%]	95	100	95	95	98	55	0
Mean total length [mm]	17.7	18.1	18.5	19.0	18.6	18.7	-- [2]
Mean wet weight [mg]	85.0	85.5	97.5	98.6	98.3	108.7	-- [2]
Mean dry weight [mg]	20.4	20.8	21.8	23.8	22.3	23.2	-- [2]
Endpoint [mg pyraclostrobin/L] (mean measured)							
$NOEC_{overall}$ (36 d)	0.0108						

[1] at 0.0240 and 0.0445 mg a.s./L hatch was complete on day 5.
[2] not determined; no fish survived at the concentration above 0.0240 mg a.s./L.

Validity criteria according to OECD TG 210 (2013)	**Obtained in this study**
The dissolved oxygen concentration should be >60% of the air saturation value throughout the test.	> 60% (5.4 – 7.9 mg/L)
The water temperature should not differ by more than ±1.5°C between test chambers or between successive days at any time during the test and should be within the temperature ranges specified for the test species (for sheepshead minnow: 25°C ±1.5°C).	Temp. range: 29.0 – 30.8°C Differences between test chambers / between successive days < 1.5°C
The analytical measure of the test concentrations is compulsory. When the measured concentrations do not remain within 80-120% of the nominal concentration, the effect concentrations should be determined and expressed relative to the arithmetic mean concentration for flow-through tests.	75.3 – 103.1% of nominal throughout the test; therefore, results are based on mean measured concentrations
Overall survival of fertilized eggs and post-hatch success in the controls and, where relevant, in the solvent controls should be greater than or equal to the limits defined (for sheepshead minnow: 75% hatching success, 80% post-hatch success).	100% hatching success and 95% post-hatch success in control; 95% hatching success and 100% post-hatch success in solvent control

Failure in terms of meeting one validity criterion is marked in **bold.**

Conclusions

In an early life stage study with sheepshead minnows (*Cyprinodon variegatus*) the overall NOEC (36 d) for pyraclostrobin was determined to be 0.0108 mg a.s./L based on mean measured concentrations.

Due to the steep dose-response curve in this study no reliable EC_{10} and EC_{20} value could be derived from this study. Overall, effects ≥ 10% only occurred at test item concentrations greater than the NOEC. Thus, the NOEC is the more conservative endpoint.

Three out of the four validity criteria of the OECD TG 210 (2013) were met. The temperatures measured

during the toxicity test were above the recommended range for this fish species. However, the individually recorded daily temperatures were always within the specified range (< 1.5°C differences) and the temperature range was similar in all treatments during the whole study conduct. Overall, the deviations are of no relevance for the overall outcome of the study.

The study is considered valid and reliable. It is relevant for classification purposes.

11.6.2 Chronic toxicity to aquatic invertebrates

11.6.2.1 Chronic toxicity to invertebrates – *Daphnia magna*

Author:	Dohmen, G.P.
Title:	Effects of BAS 500 F on mortality and reproduction of *Daphnia magna*
Date:	07.12.1999
Doc ID:	35811; Reg. Doc. # BASF 1999/11864
Guidelines:	OECD 202 Part II (21 day reproduction test considering draft revision from January 1996)
GLP:	Yes
Validity:	Acceptable
Previous evaluation:	In initial DAR (2001)

Material and Methods

Test item:	BAS 500 F (batch J.-No. 27882/191/c), purity: 97.1 %
Test species:	Waterflea (*Daphnia magna* STRAUS), neonates collected from in house culture, age at test initiation less than 24 hours.
Test design:	Semi-static test (21 d), 7 test concentrations plus control and solvent control, 10 replicates with 1 parent daphnids in each; assessment of parent mortality, offspring, and other effects at least every working day. Measuremnt of total parent length at the end of the test (d 21).
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Control, solvent control, 0.00025, 0.0005, 0.001, 0.002, 0.004, 0.008 and 0.016 mg as/L (nominal).
Test conditions:	Glass test vessels with a volume of 50 mL. M4 was used as test medium. The pH ranged between 7.92 and 8.37. The oxygen content was measured to be 8.3 – 9.3 mg/L. The total hardness was 2.26 – 2.63 mmol/L and the conductivity was 617 – 673 µS/cm. The temperature was between 20 and 21 °C. Day:night-rhythm of 16 hours light with < 1500 lux and 8 hours darkness. There was no ventilation. As food source algae were used.
Analytics:	HPLC (CP 314 method).
Statistics:	Probit analysis, Analysis of variance, Dunnett`s and Bonferroni tests

Results and Discussion

Analytical measurements: Performed in all concentrations (except for the lowest one) in fresh and aged test solutions at least weekly during the experiment period. The time weighted average test concentrations were 80% or higher of the nominal concentrations. Thus the biological results are based on nominal concentrations. The results of the chemical analysis are shown in Table 193 (extracted from study report):

Table 193: Analytical results

Nominal concentration [µg/L]	0.5	1.0	2.0	4.0	8.0	16
Time weighted average concentration [µg/L]	0.44	0.84	1.73	3.42	6.38	14.69
% of nominal	88	84	87	86	80	92

Biological results: Some parent mortality was observed at the two highest test concentrations, two dead daphnids at 0.008 mg as/L and one dead daphnid at 0.016 mg as/L. However, the numbers of dead daphnids were very low and not concentration dependent. No significant difference between treatments was observed for the onset of reproduction.

The mean number of offspring per parent in the controls was about 130. A significant reduction in the number of offspring was observed at 0.008 mg as/L and 0.016 mg as/L. No effect on reproduction was observed at concentrations of 0.004 mg as/L and less.

The two highest concentrations, 0.008 and 0.016 mg as/L, caused slightly but statistically significantly reduced growth of parent daphnids. The results are summarized in Table 194.

Table 194: Effects (21 d) of pyraclostrobin on *Daphnia magna* reproduction and parent mortality

Concentration [mg a.s./L] (nominal)	**control**	**solvent control**	**0.00025**	**0.0005**	**0.001**	**0.002**	**0.004**	**0.008**	**0.016**
Offspring/parent (21 d)	131	130	104	133	117	109	127	99 *	30 *
Parent-mortality [%] (21 d)	0	0	0	0	0	0	0	20	10
Total length [mm] (21 d)	4.48	4.53	4.47	4.45	4.36	4.34	4.45	4.10*	4.19*
Endpoints [mg pyraclostrobin/L] (nominal)									
NOEC (28 d)	0.0040								

* Statistically significant effects compared to the control

Validity criteria according to OECD TG 211	**Obtained in this study**
The mortality of the parent animals (female daphnia) does not exceed 20% at the end of the test	0%
The mean number of living offspring produced per parent animal surviving at the end of the test is ≥ 60	131 (control); 131 (solvent control)

Conclusions

In a 21-day semi-static chronic toxicity study with *Daphnia magna* the NOEC of pyraclostrobin was 0.004 mg a.s./L (nominal).

Due to the steep dose response curve, no reliable EC_{10} and EC_{20} value could be derived from this study. Overall, effects ≥ 10% only occurred at test concentrations greater than the NOEC. Thus, the NOEC is the more conservative and relevant endpoint for the risk assessment.

All validity criteria were met. The study is valid and reliable. It is relevant for classification purposes.

11.6.2.2 Chronic toxicity to invertebrates – *Americamysis bahia*

Author:	Ward, T.J. et al.
Title:	BAS 500 F: A flow-through life-cycle toxicity test with the Saltwater Mysid *Americamysis bahia*
Date:	18.02.2004
Doc ID:	7473-BA; BASF Study Number 130898, BASF Reg. Doc. No. 2004/5000004
Guidelines:	EPA, FIFRA 72-4 (b)
GLP:	Yes
Validity:	Formally valid but not acceptable for risk assessment due to apparent deficiencies; only presented for completeness
Previous evaluation:	Submitted for the purpose of renewal

Material and Methods

Test item:	BAS 500 F (batch no. WF19407), purity: 99.7%.
Test species:	Saltwater mysids (*Americamysis bahia*), juveniles, age: less than 24 hours old; source: in-house cultures, original culture obtained from "Aquatic BioSystems, Inc.", Fort Collins, Colorado, USA.
Test design:	Flow-through system (28 days); 5 test concentrations plus control and solvent control, 2 replicates per test item concentration; 30 mysids per test vessel; 15 mysids per retention chamber; on day 14 when the sex of mysids could be determined, up to ten females were segregated from the population in each glass aquarium and paired with a male in the test chambers, unpaired mysids were sexually differentiated and placed in separate test chambers; daily assessment of survival and symptoms of toxicity, assessment of reproduction (number of offspring produced by each female) from day 17 on; determination of length, dry weight and wet weight of surviving mysids at test termination.
Endpoints:	NOEC based on survival, reproductive success, length, dry and wet weight.
Test concentrations:	Control (dilution water), solvent control (0.1 mL dimethylformamide/L) and 0.28, 0.52, 1.0, 2.0 and 4.0 µg pyraclostrobin/L (nominal), corresponding to mean measured concentrations of 0.27, 0.50, 0.93, 1.9 and 3.6 µg a.s./L.
Test conditions:	20 L glass aquaria (21 cm x 40 cm x 25 cm) containing two test chambers, test volume up to 7 L; test chambers: glass petri dishes with a 12 cm high collar of Nitex screen attached (from test initiation until day 14: petri dishes of 10 cm diameter, from day 14 on: petri dishes of 6 cm diameter); dilution water: filtered, aerated, sterilized and diluted seawater; flow rate: 13.6 volume additions per 24 hours on average; salinity: 15 - 17‰; temperature: 23.0°C - 26.7°C; pH 7.5 - 8.9; oxygen content: 5.0 - 7.9 mg/L; photoperiod 16 h light : 8 h dark with a 15 minute transition period between dark and light; light intensity: approx. 38 foot candles; feeding: newly hatched *Artemia salina nauplii* three times per day, no feeding during the final 24 hours; no aeration.
Analytics:	HPLC-method with UV-detection.
Statistics:	Descriptive statistics, Bonferroni's or Williams's test for determination of NOEC values ($\alpha = 0.05$).

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at test initiation, on days 7, 14, 21, and at test termination on day 28. Measured concentrations for pyraclostrobin were between 61.5 and 100.0% of nominal at test initiation. Measured concentrations after 7, 14, and 21 days ranged from 107.1 to 125.0%, from 80.0 to 119.2% and from 83.0 to 107.7% of nominal,

respectively. At test termination, measured concentrations were between 73.1 and 85.0% of nominal. Thus the following biological results are based on mean measured concentrations. The results of the chemical analysis are shown in Table 195 (extracted from study report):

Table 195: Analytical results

Nominal Concentration of BAS 500 F (µg/L)	Measured Concentration of BAS 500 F (µg/L)						
	Study Day[1]					Mean	Percent of Nominal
	0	7	14	21	28		
Test Media							
0 (control)	ND[2]	ND	ND	ND	ND	ND	--
0 (solvent control)	ND[2]	ND	ND	ND	ND	ND	--
0.28	0.28	0.30	0.33	0.24	0.22	0.27	96
0.52	0.32	0.60[3]	0.62	0.56	0.38	0.50	96
1.0	1.0	1.1	0.98	0.83	0.76	0.93	93
2.0	1.8	2.5	1.8	1.9	1.7	1.9	95
4.0	3.5	4.4	3.2	3.4	3.4	3.6	90
Laboratory Control Spike							
1.0	1.3	1.2	1.2	1.0	0.94	1.1	110
Matrix Spike							
1.0	1.0 1.1	1.1 1.0	0.99 1.2	0.90 0.94	0.92 0.85	1.0	100
Blank							
0	ND	ND	ND	ND	ND	ND	--

[1] Pretest samples were also collected from the 0.28, 1.0, and 4.0 concentrations. The measured concentrations of BAS 500 F in these samples was 0.32, 1.1, and 3.8 µg/L.
[2] ND = not detected at or above the quantitation limit.
[3] The sample collected on day 7 had a low recovery (0.26 µg/L), possibly because the sample was collected from the incorrect test vessel. This concentration was resampled in duplicate on day 11, when the poor recovery was first observed, and the reported value is the mean of the three analyses (day 11 measured concentrations were 0.71 and 0.82 µg/L).

Biological results: The data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. Survival of unaffected (not lethargic) saltwater mysids was statistically significantly affected compared to the pooled control at the three highest test item concentrations of 0.93, 1.9 and 3.6 µg pyraclostrobin/L (Bonferroni's test / Williams's test; $\alpha = 0.05$). Reproductive success and mean total length was statistically significantly different to the pooled control data at the two highest test item concentrations (Bonferroni's test / Williams's test; $\alpha = 0.05$). Mean dry and wet weight of surviving mysids at the test item concentrations of up to and including 1.9 µg a.s./L showed no statistically significant differences compared to the pooled control data. The results are summarized in Table 196.

Table 196: Chronic toxicity (28 d) of pyraclostrobin to saltwater mysids (*Americamysis bahia*)

Concentration [µg a.s./L] (nominal)	**Control**	**Solvent control**	**0.28**	**0.52**	**1.0**	**2.0**	**4.0**
Concentration [µg a.s./L] (mean measured)	--	--	**0.27**	**0.50**	**0.93**	**1.9**	**3.6**
Survival (unaffected mysids) on day 28 [%]	88	80	83	82	73 *	52 *	0 *
Production of young per female by day 28 §	7.5	7.1	6.0	11.4	7.6	2.0 *	0 *
Mean total length on day 28 [mm]	7.4	7.3	7.4	7.2	7.3	7.1 *	-- *

Concentration [µg a.s./L] (nominal)	**Control**	**Solvent control**	**0.28**	**0.52**	**1.0**	**2.0**	**4.0**
Concentration [µg a.s./L] (mean measured)	--	--	**0.27**	**0.50**	**0.93**	**1.9**	**3.6**
Mean wet weight on day 28 [mg]	3.58	3.48	3.55	3.46	3.41	3.19	-- *
Mean dry weight on day 28 [mg]	0.74	0.76	0.78	0.76	0.77	0.75	-- *
Endpoints [µg pyraclostrobin/L] (mean measured)							
NOEC $_{overall}$ (28 d)	0.50						

§ Defined as the sum of the total number of young each day divided by the number of surviving females on the respective day
* Statistically significant differences compared to pooled control (Bonferroni's test / Williams's test; α = 0.05).

Recommendations according to EPA OPPTS 850.1350 (1996) *	**Obtained in this study**
The mortality of the parent animals does not exceed 20% at the end of the test.	12% (control) and 20% (solvent control)
The test is unacceptable if more than 25 percent of first-generation females in the control groups fail to produce young or if the average number of young produced per female in the controls is less than three.	% of first-generation females fail to produce young: no information given Mean no. of young produced per female in the controls > 3 (*i.e.* mean: 7.5)

Failure in terms of meeting one validity criterion/recommendation is marked in **bold.**
* As no concrete validity criteria are defined in the EPA guideline 850.1350 (1996), "recommendations" of the guideline have been considered for the validity check congruently with the OECD TG 211 (2012) for reproduction test with *Daphnia magna* (crustacean).

Conclusions

In a flow-through chronic toxicity study with saltwater mysids (*Americamysis bahia*), the overall NOEC (28 d) for pyraclostrobin was determined to be 0.50 µg a.s./L based on mean measured concentrations.

The most relevant recommendations were met. However, the study showed some shortcoming, *i.e.* within the study only the number of offspring was evaluated but survival, development and behaviour of the second generation (for at least 4 days, as requested by the US EPA guideline) was not reported. Additionally, the survival of male F0-generation mysids following pairing was not reported. Thus, the study did not include a full evaluation of the F0 and F1-generation. Additionally, the results were not presented separately for males and females. Overall, the test does not really present a full life-cycle test without the evaluation of the F1-generation.

The study is considered as not reliable. It is not relevant for classification purposes.

11.6.2.3 Chronic toxicity to invertebrates – *Americamysis bahia*

Author:	Dinehart, S. Anonymous (study addendum)
Title:	BAS 500 F: Life-cycle toxicity test of the saltwater mysid, *Americamysis bahia*, conducted under flow-through test conditions
Date:	01.08.2013 07.06.2019 (study addendum)
Doc ID:	68248; BASF Study Number 407434, BASF Reg. Doc. No. 2013/7002075 BASF DocID 2019/2036254 (study addendum)
Guidelines:	EPA 850.1000, EPA 850.1350, EPA 72-4

GLP:	Yes
Validity:	Acceptable
Previous evaluation:	Submitted for the purpose of renewal

Material and Methods

Test item:	BAS 500 F (batch no. CD-001236), purity: 99.02%.
Test species:	Saltwater mysid (*Americamysis bahia*), juveniles, age: less than 24 hours old; source: in-house culture.
Test design:	Flow-through system (31 d); 5 test item concentrations plus a control and a solvent control, 3 replicates for each test item concentration, the control and the solvent control, two retention baskets per test vessel: one containing mysids for reproduction observations and one for growth observations.
Endpoints:	NOEC based on survival, reproductive success, body length and dry weight.
Test concentrations:	Control (dilution water), solvent control (approximately 0.025 mL dimethylformamide/L), 0.00025, 0.00050, 0.001, 0.002, and 0.004 mg pyraclostrobin/L (nominal), corresponding to mean measured concentrations of 0.000198, 0.000365, 0.000676, 0.00128 and 0.00257 mg pyraclostrobin/L.
Test conditions:	Test vessels: glass aquaria (test volume: 20 L) with a glass pane in the middle of the tank containing two holes near the bottom; retention baskets: glass petri dish base (approximately 1.5 x 15 cm) with a nylon screen collar (mesh size: 355 µm); brood baskets: glass Petri dish base (approximately 1.5 x 10 cm) with a nylon screen collar (mesh size: 355 µm).
Analytics:	HPLC-method with UV detection.
Statistics:	Descriptive statistics; A one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test and one-tailed Williams' test were used to estimate the NOEC based on percentage of survival, growth as body length and dry weight of each individual, time to first brood, and total number of young per female. When results from Dunnett's and Williams' test were not equivalent, the more conservative result was reported. Otherwise, results from Williams' test are reported.

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at test initiation, on days 9, 14, 21, and at test termination on day 31. Measured concentrations for pyraclostrobin were between 63 and 74% of nominal at test initiation. Measured concentrations after 9, 14, and 21 days ranged from 65 to 99%, from 62 to 84% and from 53 to 71% of nominal, respectively. At test termination, measured concentrations were between 67 and 76% of nominal. Thus the following biological results are based on mean measured concentrations. The results of the chemical analysis are shown in Table 197 (extracted from study report):

Table 197: Analytical results

Measured BAS 500 F Concentration as µg a.i./L (Percent of Nominal/Percent of Mean Measured)										Diluter Stock Measured Concentration (160,000) [a]
Study Day	Control	Vehicle Control	Level 1 (0.250) [a]	Level 2 (0.500) [a]	Level 3 (1.00) [a]	Level 4 (2.00) [a]	Level 5 (4.00) [a]	Low Spike (0.148) [a]	High Spike (5.00) [a]	
-4	<MQL	<MQL	0.176 (70)	0.333 (67)	0.692 (69)	1.61 (81)	2.54 (64)	1.69 (114)	4.14 (83)	161,000 (101)
0	<MQL	<MQL	0.186 (74/94)	0.371 (74/102)	0.674 (67/100)	1.36 (68/106)	2.53 (63/98)	1.57 [b] (106)	5.11 (102)	161,000 (101)
9	0.415	0.143	0.247 (99/125)	0.430 (86/118)	0.759 (76/113)	1.39 (70/109)	2.60 (65/101)	3.22 [c] (218)	4.09 (82)	153,000 (96)
14	<MQL	<MQL	0.210 (84/106)	0.315 (63/86)	0.624 (62/93)	1.24 (62/97)	2.55 (64/99)	1.55 (105)	3.77 (75)	153,000 (96)
21	<MQL	<MQL	0.177 (71/89)	0.330 (66/90)	0.622 (62/93)	1.06 (53/83)	2.38 (60/93)	1.68 (114)	4.00 (80)	161,000 (101)
31	<MQL	<MQL	0.169 (68/85)	0.381 (76/104)	0.702 (70/105)	1.34 (67/105)	2.80 (70/109)	1.53 (103)	4.09 (82)	152,000 (95)
Mean Measured Concentration [d]	---	---	0.198 (79)	0.365 (73)	0.676 (68)	1.28 (64)	2.57 (64)	---	---	---

MQL: Minimal Quantifiable Limit = 0.0800 µg a.i./L

[a] Target nominal concentration of BAS 500 F in µg a.i./L.
[b] Average of duplicate re-analyses.
[c] Result confirmed by duplicate reanalysis.
[d] Day –4 values are not included in the mean calculations.

Biological results: Survival of F_0-generation mysids was statistically significantly affected compared to the control at the highest test item concentration of 2.57 µg pyraclostrobin/L after 7 days of exposure (William's test, $\alpha = 0.05$). However, this effect was considered to be not biologically relevant, because the magnitude of the difference in percent survival was very small (i.e. 100% survival in the control compared to 97% survival at 2.57 µg a.s./L). There were no statistically significant differences among survival rates of the F_0-generation after 13, 14, 21, and 31 days of exposure compared to the control. There was a statistically significant reduction in the mean number of young produced per female at the highest test item concentration compared to the control. Length of F_0-males and females exposed for 14 days, length of F_0-males exposed for 31 days and weight of F_0-females exposed for 31 days was statistically significantly reduced at the highest test item concentration (William's test, $\alpha = 0.05$). There was a slight but statistically significantly effect on the weights of F_0-males at the three highest test item concentrations (William's test, $\alpha = 0.05$). However, there was no impact on female weight and no impact on fecundity/reproductive performance (except for the highest treatment level). Therefore, though statistically significant, this impact is assumed to be not of biological relevance and thus not considered as an adverse effect. No statistically significant adverse effects on survival and growth of the F_1-generation mysids were detected in any of the test item concentrations tested. The results are summarized in Table 198.

Table 198: Chronic toxicity (31 d) of pyraclostrobin to saltwater mysids (*Americamysis bahia*)

Concentration [µg a.s./L] (nominal)			**Control**	**Solvent control**	**0.25**	**0.50**	**1.0**	**2.0**	**4.0**
Concentration [µg a.s./L] (mean measured)			--	--	**0.198**	**0.365**	**0.676**	**1.28**	**2.57**
F_0-generation	**Survival (pre-pairing)**	**survival after 7 days of exposure [%]**	100	91	99	100	98	98	97 * a)
		survival after 13 days of exposure [%]	98	88	99	97	98	93	92
	Survival (post-pairing)	**survival after 14 days of exposure [%]**	100	100	100	100	98	98	100

Concentration [µg a.s./L] (nominal)			**Control**	**Solvent control**	**0.25**	**0.50**	**1.0**	**2.0**	**4.0**
Concentration [µg a.s./L] (mean measured)			**--**	**--**	**0.198**	**0.365**	**0.676**	**1.28**	**2.57**
		survival after 21 days of exposure [%]	98	100	100	100	98	98	100
		survival after 31 days of exposure [%]	97	98	100	93	93	90	100
	Growth	**length of males exposed for 31 days [mm]** [#]	6.11 ± 0.122	6.14 ± 0.0709	6.08 ± 0.0842	6.19 ± 0.114	5.95 ± 0.132	6.01 ± 0.206	5.83 ± 0.193 *
		length of females exposed for 31 days [mm] [#]	6.23 ± 0.0515	6.32 ± 0.150	6.44 ± 0.0565	6.45 ± 0.118	6.08 ± 0.384	6.26 ± 0.118	5.98 ± 0.149
		weight of males exposed for 31 days [mg] [#]	1.14 ± 0.121	1.07 ± 0.020	1.07 ± 0.900	1.12 ± 0.0402	0.989 ± 0.0939 *	0.972 ± 0.0812 *	0.937 ± 0.0982 *
		weight of females exposed for 31 days [mg] [#]	1.51 ± 0.141	1.44 ± 0.0336	1.39 ± 0.108	1.46 ± 0.110	1.30 ± 0.283	1.37 ± 0.146	1.01 ± 0.109 *
	Reproduction	**days to first brood release** [#]	17.9 ± 2.15	18.2 ± 1.10	17.7 ± 0.850 [b)]	18.5 ± 3.32	19.0 ± 3.37	21.8 ± 4.41	17.0
		mean young per female [#, c)]	12.8 ± 4.33	14.1 ± 7.64	3.19 ± 0.577 [b)]	11.3 ± 0.247	14.6 ± 11.5	8.00 ± 6.61	0.191 ± 0.330 *
F_1-generation	**Survival**	**survival after 4 days of exposure [%]**	100	100	100	100	96	88	100
		survival after 7 days of exposure [%]	100	98	100	98	96	88	100
		survival after 10 days of exposure [%]	100	93	100	98	96	88	100
	Growth	**length of males exposed for 10 days [mm]** [#]	4.17 ± 0.137	4.41 ± 0.0782	4.43 ± 0.0382	4.48 ± 0.154	4.39 ± 0.0531	4.30 ± 0.247	4.29
		length of females exposed for 10 days [mm] [#]	4.18 ± 0.0566	4.57 ± 0.206	4.68 ± 0.0487	4.69 ± 0.194	4.42 ± 0.174	4.53 ± 0.209	4.34
Endpoint [µg pyraclostrobin/L] (mean measured)									
$NOEC_{reproduction}$ (31 d)			1.28						
$NOEC_{male\ weight}$ (31 d)			0.365						

Concentration [µg a.s./L] (nominal)	**Control**	**Solvent control**	**0.25**	**0.50**	**1.0**	**2.0**	**4.0**
Concentration [µg a.s./L] (mean measured)	--	--	**0.198**	**0.365**	**0.676**	**1.28**	**2.57**
EC_{10} /EC_{20} (based on dry weight of male mysids after 31 d)	1.514 / 2.57 (n.r. [+])						

* Statistically significant differences compared to the control (William's test, $\alpha = 0.05$).
[#] Values represent mean and standard deviation from all replicates.
[+] n.r. = no reliable EC_x values could be calculated
[a)] The statistically significant difference in F0-mysid survival rate in the 2.57 µg/L treatment on day 7 was judged not to be biologically meaningful because of the magnitude of the difference in mean percent survival between the control and the solvent control.
[b)] The 0.198 µg/L treatment was excluded from statistical analyses for reproduction endpoints because of low reproductive output from F_0-female mysids in this treatment which did not appear to reflect a test substance treatment-related effect.
[c)] Paired females that produced no young were excluded from the mean calculation.

Conclusions

The NOEC (31 d) based on female weight and reproduction for pyraclostrobin was determined to be 0.00128 mg a.s./L based on mean measured concentrations. Based on the significant effect on the weight of males the NOEC would be 0.000365 mg a.s./L (mean measured). A biological relevance of this effect cannot be excluded. Even if there are only slight effects, there is a dose-reponse relationship for increasing concentrations. Further more it is not known if a reduced weight may has an impact on the fitness of the males. Thus, the NOEC of 0.000365 mg a.s./L is considered as relevant endpoint for the risk assessment.

The calculation of EC_{10} and EC_{20} values does not deliver reliable endpoints due to big range of confidence limits and less than 20% effect at all. However, the values are still presented for this study summary. 31-d EC_{10} for dry weight of male mysids (most sensitive parameter) based on mean measured concentrations was 0.001514 mg a.s./L (95% CL: 0.000128 – 0.0029 mg/L).

The study is considered valid and reliable. It is relevant for classification purposes.

11.6.2.4 Chronic toxicity to invertebrates – *Chironomus riparius*

Author:	Dohmen, G.P.
Title:	Effects of BAS 500 F on the development of sediment dwelling larvae of *Chironomus riparius* in a water-sediment system
Date:	20.01.2000
Doc ID:	35966; BASF DocID 2000/1000010
Guidelines:	BBA-guideline proposal: 'Effects of plant protection products on the development of sediment dwelling larvae of Chironomus riparius in a water-sediment system.", Mitteilungen aus der Biol. Bundesanstalt, Heft 315, Blackwell Berlin, 1995, pp 70-84.
GLP:	Yes
Validity:	Formally valid, but no reliable endpoint can be derived. Additional information only.
Previous evaluation:	In initial DAR (2001)

Material and Methods

Test item:	BAS 500 F, unlabelled: purity: 97.1 %; 14C-labelled BAS 500 F, purity 98.0 %
Test species:	*Chironomus riparius* Meigen, egg masses obtained from in-house cultures, larvae < 3 days at test initiation.

Test design:	Static system (28 days); 25 larvae per test vessel, 3 replicates per concentration plus a control with 2 and a solvent control with 4 replicates; assessment of emergence rate and development rate.
Endpoints:	NOEC, EC_{10} and EC_{50} (regarding emergence rate and development rate).
Test concentrations:	Control, solvent control, 0.020, 0.040, 0.080, 0.160, 0.320 mg as/L (nominal)
Test conditions:	Glass vessels, about 2 cm sediment layer (soil according to OECD TG 207), 1.8 L "M4" water (Elendt medium) according to a 16.5 cm water layer, pH 7.76-8.09, oxygen content 8.2-9.0 mg/L, total hardness 2.45 mmol/L, conductivity 683 µS/cm (all at test initiation, hardness and conductivity from a combined sample), feeding with TetraMin, slight ventilation, mean water temperature 20.8 + 1.4 °C, light intensity 680 - 1570 lux, day: night-rhythm 16:8 h.
Analytics:	Analytical verification of the test concentrations were conducted using reversed phase HPLC and UV-detection. Radio-labelled compound was analyzed using LSC and HPTLC.
Statistics:	Descriptive statistics; probit analysis for determination of EC_x values; ANOVA followed by Dunnett's test, Bonferroni-test and/or and Williams-test for determination of the NOEC values.

Results and Discussion

Analytical measurements: Analytical measurements of test substance shortly after addition to the system yielded recoveries in the water phase of 98.5 to 105.7% of the nominal concentrations, indicating the correct addition of test substance to the system. As was to be expected from the water sediment study, the test substance dissipated rapidly from the water phase, a respective transient increase was found in the sediment. Pore water concentrations were negligible during the whole experiment. The following biological results are based on nominal concentrations.

Measurements of radioactivity in the water phase for the 0.08 and 0.32 mg a.s./L treatment are shown in Table 199 (Extracted from the study report). Distribution of radioactivity and material balance for the 0.08 mg/L and 0.32 mg/L treatment in shown in Table 200 and Table 201, respectively (extracted from study report):

Table 199: Analytical results

	0.08 mg/l			0.32 mg/l		
DAT	vessel No.	ug total	%TAR	vessel No.	ug total	%TAR
0	13	135.077	**93.8**	19	534.450	**92.8**
	14	137.960	**95.8**	20	559.033	**97.1**
	15	177.203	**123.1**	21	542.502	**94.2**
	pooled sample	143.424	**99.6**	pooled sample	567.504	**98.5**
	vessel 1d	150.096	**104.2**	vessel 1d	545.821	**94.8**
	vessel 7d	139.706	**97.0**	vessel 7d	554.509	**96.3**
1	13	n.m.		19	n.m.	
	14	n.m.		20	n.m.	
	15	n.m.		21	n.m.	
	pooled sample	n.m.		pooled sample	n.m.	
	vessel 1d	135.476	**94.1**	vessel 1d	415.717	**72.2**
	vessel 7d	n.m.		vessel 7d	n.m.	
2	13	125.519	**87.2**	19	258.549	**44.9**
	14	90.657	**63.0**	20	413.154	**71.7**
	15	126.303	**87.7**	21	333.718	**57.9**
	pooled sample	106.776	**74.2**	pooled sample	317.484	**55.1**
	vessel 7d	69.023	**47.9**	vessel 7d	319.755	**55.5**
7	13	50.235	**34.9**	19	104.743	**18.2**
	14	24.915	**17.3**	20	156.794	**27.2**
	15	34.801	**24.2**	21	145.872	**25.3**
	pooled sample	n.m.		pooled sample	n.m.	
	vessel 7d	30.793	**21.4**	vessel 7d	144.658	**25.1**
21	13	17.761	**12.3**	19	38.542	**6.7**
	14	10.324	**7.2**	20	67.003	**11.6**
	15	12.102	**8.4**	21	48.038	**8.3**
	pooled sample	12.060	**8.4**	pooled sample	47.412	**8.2**
28	13	16.541	**11.5**	19	41.287	**7.2**
	14	8.742	**6.1**	20	62.525	**10.9**
	15	10.524	**7.3**	21	36.508	**6.3**

DAT days after treatment
n.m. not measured
TAR total applied radioactivity

Table 200: Distribution of radioactivity and material balance fo 0.08 mg/L treatment

DAT	vessel No.	% TAR					
		water	sediment				material balance
			ERR	RRR	pore water	total	
1	vessel 1d	94.1	10.1	0.3	0.0	10.4	104.5
7	vessel 7d	21.4	74.7	2.1	0.0	76.8	98.2
28	13	11.5	73.2	9.2	0.1	82.4	93.9

DAT = days after treatment
ERR = extractable radioactive residues
RRR = residual radioactive residues (bound residues)
TAR = total applied radioactivity (100% = 144ug/test vessel)

Table 201: Distribution of radioactivity and material balance fo 0.32 mg/L treatment

DAT	vessel No.	% TAR					
		water	sediment				material balance
			ERR	RRR	pore water	total	
1	vessel 1d	72.2	26.4	0.8	0.0	27.2	99.4
7	vessel 7d	25.1	69.1	2.1	0.0	71.2	96.3
28	20	10.9	70.6	11.0	0.0	81.7	92.6

DAT = days after treatment
ERR = extractable radioactive residues
RRR = residual radioactive residues (bound residues)
TAR = total applied radioactivity (100% = 576ug/test vessel)

Biological results: The emergence rates were generally quite high. More than 70% - the required minimum for the untreated controls - of the chironomids emerged in all but the highest test concentration and more than 90% emergence was observed in the controls and concentrations up to 0.080 mg/L. Statistically significant effects on the emergence rate were only found at the highest test concentrations, 0.160 and 0.320 mg/L (Williams Multiple t-test, $\alpha = 0.05$). The latter caused a nearly 50% reduction in emergence. Statistically significant differences between the overall development rates of the treatments and the controls were found at the two highest test concentrations, respectively the three highest concentrations (Williams Multiple t-test, $\alpha = 0.05$). Results are summarized in Table 202.

Table 202: Effects of pyraclostrobin on emergence and development of *Chironomus riparius*

Concentration [mg a.s./L (nominal)	**Control**	**Solvent control**	**0.020**	**0.040**	**0.080**	**0.160**	**0.320**
Emergence rate (ER) [% emerged midges]	0.95	1.00	0.93	0.93	0.91	0.80 **	0.56 *
Development rate per day (DR)	0.077	0.075	0.074	0.074	0.069 **	0.065 *	0.056 *
Endpoints [mg pyraclostrobin/L] (nominal)							
$EC_{50emergence\ rate}$ (28 d)	0.377 (95% confidence limits: 0.302 – 0.451)						
$EC_{10\ emergence\ rate}$ (28 d)	0.129 (95% confidence limits: 0.107 – 0.151)						
$NOEC_{emergence\ rate}$ (28 d)	0.080						

$NOEC_{development\ rate}$ (28 d)	0.040

n.d. = not determined
* Statistically significant difference compared to the pooled control (Dunnett's test and Bonferroni-test and Williams-test, α = 0.05).
** Statistically significant difference compared to the pooled control (Williams-test, α = 0.05).

Validity criteria according to OECD TG 219 (2004)	**Obtained in this study**
The emergence in the controls must be at least 70% at the end of test.	95% (control) and 100% solvent control)
C. riparius emergence to adults from control vessels should occur between 12 and 23 days after their insertion into the vessels.	between day 12 and 23 after insertion (except for minor deviation: 1 – 2 animals which emerged on day 24 in the controls)
At the end of test, pH and dissolved oxygen concentration should be measured in each vessel: oxygen concentration ≥60% of air saturation value at the temperature used, the pH of overlying water should be in the 6-9 range in all test vessels.	O_2: > 60% (8.2 – 9.0 mg/L) pH: 7.8 – 8.1
The water temperature should not differ by more than ±1.0°C	Mean water temperature 20.8 **± 1.4 °C**

Conclusions

In a 28-day static sediment test with *Chironomus riparius* the NOEC values of pyraclostrobin were determined to be 0.080 mg a.s./L based on emergence rate and 0.040 mg a.s./L based on development rate (nominal). The EC_{10} based on emergence rate was 0.129 mg a.s./L (nominal).

The minor deviation from the recommendations for the variation of water temperatures is of no relevance for the overall outcome of the study. Endpoints should be based on mean measured concentrations because the concentrations were not maintained in the range of +/- 20% of nominal over the test duration. The analytical results showed that pyraclostrobin concentrations in the water phase clearly decreased over time while sediment concentrations increased. Thus, it is not acceptable to base the endpoints on nominal water concentrations. As only the 0.08 and 0.32 mg a.s./L treatments, i.e. concentrations higher as the NOEC, were analytically verified it is not possible to calculate mean measured endpoints. Considering the analytical results the RMS concludes that no fully reliable endpoint could be derived from the study. However, as this study indicates, that there was a transfer from the water phase into the sediment, the available spiked sediment tests should be considered for the risk assessment. For the exposure via the water phase it can be assumed that chironomids are covered by the assessment based on the clearly more sensitive aquatic invertebrate *Americamysis bahia*.

The study is regarded as additional information and is not reliable. It is not relevant for classification purposes.

11.6.2.5 Chronic toxicity to invertebrates – *Chironomus riparius*

Author:	Kuhl, R. and Wydra, V.
Title:	Effects of BAS 500 F (Pyraclostrobin) on the development of sediment dwelling larvae of *Chironomus riparius* in a sediment-water system - Exposed via spiked sediment
Date:	06.03.2013
Doc ID:	74981250; DocID 2012/1185699
Guidelines:	OECD 218 (2004)
GLP:	Yes

Validity:	Acceptable
Previous evaluation:	Submitted for the purpose of renewal

Material and Methods

Test item:	BAS 500 F (batch no. 10-510009), purity: 100 %
Test species:	Non-biting midge (*Chironomus riparius*), first instar larvae, 3 days old at test initiation; source: in-house culture.
Test design:	Static system (28 days); 5 test concentrations plus a solvent (acetone) control and a water control, 4 replicates per test item concentration and for the water control, 6 replicates for the solvent control; 20 larvae were added to each test vessel; assessment of emergence rate and development rate.
Endpoints:	NOEC, EC_{10}, EC_{20} and EC_{50} (regarding emergence rate and development rate).
Test concentrations:	Solvent (acetone) control, water control, 0.3, 0.6, 1.2, 2.4 and 4.8 mg a.s./kg dry sediment (nominal), corresponding to mean measured concentrations of 0.66, 1.37 and 2.83 mg a.s./kg dry sediment in the 1.2, 2.4 and 4.8 a.s./kg dry sediment treatments.
Test conditions:	600 mL glass vessels with 100 g spiked wet artificial sediment (according to OECD 218), 400 mL M4 water (Elendt medium) corresponding to a water layer of about 6.4 cm; pH 7.6 - 8.6; oxygen 62% to 100%; total hardness: 284.8 - 320.4 mg $CaCO_3$/L at test initiation and 311.5 - 329.3 mg $CaCO_3$/L at test termination; conductivity: 588 µS/cm; ammonia: 1.2 mg/L at test initiation and 2.0 mg/L at test termination; water temperature: 20°C - 21°C; light intensity: 620 - 720 lux; photoperiod: 16 h light : 8 h dark; continuous gentle aeration; food: TetraMin (days 0-10: 0.5 mg food/larva/day, days 11-27: 0.5 - 1.0 mg food/larva/day).
Analytics:	LC-MS/MS-method
Statistics:	Descriptive statistics, Student-t-test ($p < 0.05$) for comparison of the emergence and development rates in the control groups; ANOVA followed by one-sided Williams' t-test for determination of the NOEC based on emergence and development rate ($\alpha = 0.05$).

Results and Discussion

Analytical measurements: Analytical verification of test item concentrations in the overlying water, the pore water and the sediment was conducted in the test concentrations of 1.2 and 4.8 mg a.s./kg dry sediment at the beginning and the end of the test. Recoveries in the sediment were in a range between 86 and 101% of the nominal concentrations at test initiation, and < LOQ (limit of quantification = 0.57 mg a.s./kg dry sediment) and 17% of nominal at test termination. Pyraclostrobin concentrations found in the overlaying water ranged from < LOQ (LOQ = 0.001 mg/L) to 0.4% of nominal concentrations on day 0 and from < LOQ to 0.03% of nominal on day 28. Measured pore water concentrations were between 0.2% and 0.9% of nominal on test start and < LOQ (LOQ = 0.001 mg/L) in both test treatments on test end. The following biological results are based on nominal sediment concentrations. Additionally, biological endpoints based on mean measured values are given. The results of the chemical analysis are shown in Table 203 and Table 204 (extracted from study report):

Table 203: Analytical results - sediment

sample description nominal [mg test item/ kg d.s.]	day	test item found [µg/L][1]	D.F.	calculated test item [µg/L][1]	mass calculated [µg][2]	mass nominal [µg][3]	[mg test item/ kg d.s.]	% of nominal[4]
control	0	0.506	1	0.506	0.238	0.000	0.003	<LOQ
solvent control	0	0.813	1	0.813	0.405	0.000	0.006	<LOQ
1.2	0	13.799	10	137.991	69.317	83.280	0.998	83
1.2	0	14.834	10	148.336	73.220	83.280	1.054	88
4.8	0	14.277	50	713.828	352.816	333.120	5.081	106
4.8	0	12.685	50	634.256	317.924	333.120	4.578	95
control	28	<LoD	1	n.a.	n.a.	0.000	n.a.	n.a.
solvent control	28	<LoD	1	n.a.	n.a.	0.000	n.a.	n.a.
1.2	28	2.942	10	29.420	15.679	83.280	0.226	<LOQ
1.2	28	2.618	10	26.178	13.860	83.280	0.200	<LOQ
4.8	28	2.484	50	124.215	64.986	333.120	0.936	20
4.8	28	1.817	50	90.839	47.870	333.120	0.689	14

[1] The tabulated results represent results calculated on the exact raw data

[2] Recalculated to the mass of test item in the sediment of a complete test vessel

[3] Nominal amount of test item given to one test vessel

[4] The results represent rounded values

LoD: Limit of Detection = 0.012 µg test item/L

LoQ: Limit of Quantification = 0.57 mg test item/kg d.s.

n.a. not applicable

d.s. dry sediment

D.F. Dilution factor

Table 204: Analytical results – overlying and pore water

sample description nominal [mg test item/ kg d.s.]	day	test item found [µg/L][1]	D.F.	calculated test item [µg/L][1]
Overlying water:				
control	0	0.238	5	< LoQ_{DIN}
solvent control	0	0.013	5	< LoQ_{DIN}
1.2	0	0.980	5	< LoQ_{DIN}
1.2	0	1.012	5	< LoQ_{DIN}
4.8	0	4.060	5	20.298
4.8	0	4.076	5	20.378
Pore Water:				
control	0	<LoD	5	n.a.
solvent control	0	0.076	5	< LoQ_{DIN}
1.2	0	1.895	5	9.476
1.2	0	1.808	5	< LoQ_{DIN}
4.8	0	8.229	5	41.146
4.8	0	9.264	5	46.318
Overlying water:				
control	28	<LoD	5	n.a.
solvent control	28	<LoD	5	n.a.
1.2	28	0.165	5	<LoQ
1.2	28	0.015	5	<LoQ
4.8	28	0.211	5	1.055
4.8	28	0.264	5	1.322
Pore Water:				
control	28	<LoD	5	n.a.
solvent control	28	<LoD	5	n.a.
1.2	28	0.034	5	<LoQ
1.2	28	0.024	5	<LoQ
4.8	28	0.146	5	<LoQ
4.8	28	0.113	5	<LoQ

[1] The tabulated results represent results calculated on the exact raw data

LoD: Limit of Detection = 0.012 µg test item/L

LoQ_{DIN}: Limit of Quantification = 1.83 µg test item/L

LoQ: Limit of Quantification = 1 µg test item/L

n.a. not applicable

d.s. dry sediment

D.F. Dilution factor

Biological results: In the control and the solvent control mean emergence rates of 78.8 % and 71.7 % and mean development rates of 0.057 and 0.055 were observed, respectively. In the test item concentrations of up to and including 4.8 mg a.s./kg dry sediment, between 60% and 76% of the test animals emerged until day 28. No statistically significant difference was observed between the controls (Student-t-test, $p < 0.05$). Hence, the controls were pooled and used as the reference in all evaluations. Statistically significant differences compared to the pooled control were found for the emergence rates at the highest test item concentration (Williams Multiple t-test, $\alpha = 0.05$). No statistically significant effect on the development was observed in any treatment group (Williams Multiple t-test, $\alpha = 0.05$). The results are summarized in Table 205.

Table 205: Effects of pyraclostrobin on emergence and development of *Chironomus riparius*

Concentration [mg a.s./kg dry sediment] (nominal)	**Control**	**Solvent control**	**0.3**	**0.6**	**1.2**	**2.4**	**4.8**
Emergence rate (ER) [% emerged midges] [#]	78.8 ± 19.7	71.7 ± 11.7	66.3 ± 13.1	75.0 ± 4.1	73.8 ± 12.5	76.3 ± 9.5	60.0 * ± 10.8
Development rate per day (DR) [#]	0.057 ± 0.001	0.055 ± 0.001	0.057 ± 0.003	0.056 ± 0.001	0.056 ± 0.002	0.059 ± 0.003	0.058 ± 0.002
Endpoints [mg pyraclostrobin/kg dry sediment] (nominal)							
$EC_{50\ emergence\ rate}$ (28 d)	> 4.8 (mean measured: > 2.83)						
$EC_{20\ emergence\ rate}$ (28 d)	> 4.8 (mean measured: > 2.83)						
$EC_{10\ emergence\ rate}$ (28 d)	> 4.8 (mean measured: > 2.83)						
$NOEC_{emergence\ rate}$ (28 d)	2.4 (mean measured: 1.37)						
$NOEC_{development\ rate}$ (28 d)	≥ 4.8 (mean measured: ≥ 2.83)						

[#] Values represent mean and standard deviation from all replicates, each with 20 larvae.
* Statistically significant difference compared to the pooled control (Williams Multiple t-test, α = 0.05).

Validity criteria according to OECD TG 218 (2004)	**Obtained in this study**
The emergence in the controls must be at least 70% at the end of test.	78.8 and 71.7% (control and solvent control, respectively)
C. riparius emergence to adults from control vessels should occur between 12 and 23 days after their insertion into the vessels.	between day 16 and 20 after insertion
At the end of test, pH and dissolved oxygen concentration should be measured in each vessel: oxygen concentration ≥60% of air saturation value at the temperature used, the pH of overlying water should be in the 6-9 range in all test vessels.	O_2: 62 – 100% pH: 8.3 – 8.6
The water temperature should not differ by more than ±1.0°C	20 - 21°C

Conclusions

In a 28-day static sediment test with *Chironomus riparius* the NOEC values of pyraclostrobin were determined to be 2.4 mg a.s./kg dry sediment (nominal; equivalent to the mean measured concentration of 1.37 mg a.s./kg dry sediment) based on emergence rate and ≥ 4.8 mg a.s./kg dry sediment (nominal; equivalent to the mean measured concentration of ≥ 2.83 mg a.s./kg dry sediment) based on development rate. The EC_{10} and EC_{20} value based on emergence rate were both > 4.8 mg a.s./kg dry sediment (nominal; equivalent to the mean measured concentration of > 2.83 mg a.s./kg dry sediment). Based on the analytical results the endpoints have to be based on mean measured concentartions. The study if formally valid. However, it has to be highlighted that only the concentrations of the 1.2 and 4.8 mg/kg dry sediment treatments were analytically verified. As all endpoints were in the range of the analytically measured treatments this can be accepted. However, it is unclear how the mean measured concentrations were calculated for the 2.4 mg/kg sediment treatment as no analytical measuremnts were perfomed for this treatment. It is assumed that the mean measured was based on the recovery at the beginning and the end measured in the 4.8 mg/kg treatment. Overall the suitability of the analytical verification may be questioned but considering that chironomids are not among the most sensitive aquatic invertebrates the results can be accepted.

The study is considered reliable with restrictions. It is relevant for classification purposes.

11.6.3 Chronic toxicity to algae or other aquatic plants

Study summaries are already shown in chapter 11.5.3.

11.6.4 Chronic toxicity to other aquatic organisms

No information available.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Acute aquatic toxicity data is available for fish, invertebrates, algae and aquatic plants. The lowest reliable acute endpoints per organism group were for *Oncorhynchus mykiss*: LC_{50} (96 h) = 0.00616 mg a.s./L, *Americamysis bahia*: LC_{50} (96 h) = 0.00416 mg a.s./L, *Navicula pelliculosa*: E_rC_{50} (72 h) = 0.011 mg a.s./L and *Lemna gibba*: E_rC_{50}/E_yC_{50} (14 d) > 1.077 mg a.s./L. Toxicity to aquatic organisms of three trophic levels (fish, crustaceans and algae) is below 1 mg/L (EC_{50} < 1 mg/L). Therefore, pyraclostrobin fulfils the classification criteria for Aquatic Acute 1, with an M-factor of 100 (0.001 < L(E)C50 ≤ 0.01 mg/L) based on acute toxicity of invertebrate *Americamysis bahia*.

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

Chronic aquatic toxicity data is available for fish, invertebrates, algae and aquatic plants. The lowest reliable chronic endpoints per organism group were for *Oncorhynchus mykiss*: NOEC (98 d) = 0.00235 mg/L, *Americamysis bahia*: NOEC (31 d) = 0.000365 mg/L, *Navicula pelliculosa*: NOEC (120 h) = 0.0018 mg/L, and *Lemna gibba*: E_rC_{10} (14 d) = 0.82 mg a.s./L.

Based on log Kow = 3.99 and BCF (*Lepomis macrochirus*) = 776 in fish, pyraclostrobin is considered to possess the potential to bioconcentrate for classification purposes, indicated by experimental BCF ≥ 500.

For classification purpose it is applicable to classify pyraclostrobin as not readily degradable (< 70 % degradation within 28 days) according to the ready biodegradability test OECD 301F that showed 0-10 percentage degradation after 28 days.

In a study on aerobic mineralisation in surface water (OECD 309) it was shown that pyraclostrobin was slowly degradable, with a whole system $DegT_{50}$ = 26.4 – 28.3 d and water $DissT_{50}$ = 410 – 458 d (half-life > 16 d). In a water-sediment study with two test systems (OECD 308) it was also shown that pyraclostrobin was slowly degradable, with a whole system $DegT_{50}$ = 25 d, water $DissT_{50}$ = 3.6 d and sediment $DegT_{50}$ = 27.7 d.

Also, the abiotic degradation due to hydrolysis showed that pyraclostrobin is stable at pH 5 and 7 and very slow degradation at pH 9 and 25 °C.

According to results of biotic and abiotic degradation studies pyraclostrobin is considered as not rapidly degradable in the aquatic environment.

Chronic toxicity to aquatic organisms of three trophic levels (fish, invertebrates and algae) is below 0.1 mg/L (NOEC or EC_{10} < 0.1 mg/L). Furthermore, the substance has a high bioaccumulation potential and is not rapidly degradable. Therefore, pyraclostrobin fulfils the criteria for classification as Aquatic Chronic 1, with M-factor 100 (0.0001 < NOEC ≤ 0.001 mg/L) based on chronic toxicity to invertebrate *Americamysis bahia*.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Pyraclostrobin can be classified as Aquatic Acute 1, H400, with an M-factor of 100.

Pyraclostrobin can be classified as Aquatic Chronic 1, H410, with M-factor 100.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Pyraclostrobin is an active substance in plant protection products. In addition to its fungicidal effects, pyraclostrobin shows also physiological effects leading to further increased yield and quality as well as improved tolerance against biotic and abiotic stress in many crops. The substance is currently included in Annex VI of Regulation (EC) No 1272/2008 with classification as Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410) with M-factor of 100 for both hazard classes.

The DS proposed to classify the substance as:

- **Aquatic Acute 1 (H400) with M-factor of 100** based on a 96h EC_{50} value of 0.00416 mg/L for the invertebrate *Americamysis bahia*.
- **Aquatic Chronic 1 (H410) with M-factor of 100** based on 31d NOEC value of 0.000365 mg/L for the invertebrate *Americamysis bahia.* The substance has a high bioaccumulation potential and is not rapidly degradable.

Degradation

A hydrolysis study according to OECD TG 111 and EPA 161-1 and in compliance with GLP was run at pH 4, 5, 7 and 9 and at 50 °C and at pH 5, 7 and 9 and 25 °C in the dark in sterile aqueous buffered solutions. Hydrolysis was pH dependent. The substance is stable at pH 5 and 7. Under alkaline conditions (pH 9) a very slow degradation of the substance was observed at 25 °C. Only at high temperatures (50 °C) and under alkaline conditions (pH 9) a comparatively faster degradation was observed, but this does not represent common environmental conditions.

The study on direct aqueous photolysis of pyraclostrobin in sterile aqueous buffered solutions at pH 5 at 22±1 °C was conducted according to BBA IV, 6-1, OECD Draft Test Guideline "Photo-transformation of Chemicals in Water" Part A. During direct photolysis, a very fast degradation of the substance was observed. A large number of degradation and rearrangement products occurred, only some of them being stable under simulated environmental conditions. The molar mass and/or structure of 33 metabolites could be determined. Five of the metabolites (BF 500-11, BF 500-13, BF 500-14, BF 500-15 and 500M58) occurred once or several times with amounts >10 % TAR. Mineralisation after 25 days for two labels were 22% TAR and 4% TAR. In the dark control, no degradation was observed. The photolytic half-life for pyraclostrobin was calculated to be 0.06 days under study conditions. The quantum yield of pyraclostrobin was estimated to be 0.217.

One ready biodegradability test was available according to EEC 92/69, OECD TG 301F and ISO 9408 and compliance with GLP. The degree of biodegradation (% biological oxygen

demand/theoretical oxygen demand) over the 28-day test duration was in the range 0-10%. The substance is therefore not readily biodegradable under test conditions.

In an aerobic mineralisation in surface water study performed according to OECD TG 309 the degradation of pyraclostrobin was studied in a pelagic and suspended solid test under aerobic conditions for 63 days at 20 °C in the dark. In the pelagic test, pyraclostrobin was degraded very slowly. More than 78–97% TAR was still detectable as unchanged parent after 63 days. The degradation of pyraclostrobin was characterized by slow hydrolysis and formation of low amounts of cleavage products, of which BF 500-5 occurred at 5.6-10.9% TAR. All other peaks never exceeded 2.1% TAR. In the suspended solid test, pyraclostrobin dissipated quickly from the water phase (< 45% TAR after 3 days) and adsorbed to the solid particles floating in the water. In the water phase of the suspended solid test, the hydrolysis product BF 500-5 (7.7% TAR), unidentified substance (5.8% TAR) and others (≤ 3.8% TAR) were detected. In the sediment extracts of the suspended solid test, the following metabolites were formed: BF 500-3, BF 500-6 (≤ 2.3% TAR), BF 500-7 (≤ 2.6% TAR) and other components (≤ 0.5% TAR). The formation of volatiles in both test variants was generally low (< 5% TAR), irrespective of test concentration or label position. Overall, the degradation of pyraclostrobin was characterized by a low mineralization rate in both test variants irrespective of test concentration or label position. The amount of $^{14}CO_2$ never exceeded 5% TAR within 63 days. The $DegT_{50}$ values in the pelagic test ranged from 410 to 458 days for pyraclostrobin and from 11 to 29 days for BF 500-3. In the suspended solid test, the $DegT_{50}$ for pyraclostrobin for the whole system ranged from 26 to 28 days, while the $DisT_{50}$ for the water compartment range from 7 to 10 days and for the suspended sediment from 44 to 47 days. The $DisT_{50}$ for BF 500-5 was 103 days when calculated with the high test concentration. For the low test concentration, no adequate fit was achieved. In general, pyraclostrobin hydrolyses only slowly under the pelagic test conditions, but adsorbs quickly to suspended solids, when available, and is then further degraded by formation of bound residues.

The distribution and degradation of radiolabeled pyraclostrobin was studied in two sediment-water systems, taken from a pond (System A) and a pond-like side arm of a river (System B) up to 100 days in dark. The study was conducted according to the SETAC Europe, BBA IV, 5-1, US-EPA, Subdivision N, 162-4 (dark study). The radioactivity moved quite fast from the water to the sediment. The radioactivity in the water decreased to less than 25% TAR within 7 days in system A and within 2 days in system B. A further decrease to less than 3% TAR after 100 days was observed in both systems. In the sediment a corresponding increase was seen which accounted for more than 90% TAR at the end of the incubation period. Low mineralization was observed in both test systems and accounted at a maximum of 4.6% of applied radioactivity and no other volatile degradants were detected. High amounts of bound residues were formed in the sediment which accounted for up to 61.8% TAR in system A and 54.1% TAR in system B. No detectable amounts of pyraclostrobin were released from the bound residues. The following three metabolites were identified in the study: BF 500-3 accounted for a maximum of 67.6% AR at day 14 (1.9% in water, 65.7% in sediment, System B), BF 500-6 accounted for a maximum of 6.5% AR at days 61 and 100 (only in sediment, System A) and BF 500-7 accounted for a maximum of 6.3% AR at day 61 (only in sediment, System A, only once > 5%). The kinetic evaluation of the study following SFO kinetics indicated that pyraclostrobin degraded with

DT_{50} values for the whole system of 23.3 days and 26.8 days and DT_{90} values for the whole system of 77.4 days and 89.1 days for the two labels in system A. The geometric mean DT_{50} value is 25.0 days. No reliable endpoints could be derived for System B. The dissipation from the water phase followed biphasic kinetics in both systems, with $DissT_{50}$ < 3 days and $DissT_{90}$ < 30 days. The dissipation from the sediment followed SFO in system A, with $DissT_{50}$ < 30 days and $DissT_{90}$ < 100 days.

The DS concluded that pyraclostrobin is considered to be not rapidly degradable in the aquatic environment, for classification and labelling purposes.

Bioaccumulation

For pyraclostrobin, the measured octanol-water partition coefficient (log P_{OW}) determined according to OECD TG 117 (HPLC method) was 3.99. The effect of pH was not investigated since there was no dissociation in water.

In a study performed according to OECD TG 305 and EPA 165-4 the bluegill sunfish (*Lepomis macrochirus*) were tested in a flow-through system at nominal concentration of 300 µg/L of ^{14}C-pyraclostrobin for 37 days followed by a 14-day depuration period for the chlorphenyl label and a 21-day phase for the tolyl label. The bioconcentration factor (BCF) for whole fish normalized for a lipid content of 5% was 712 for the chlorphenyl label and 776 for the tolyl label.

The DS concluded that pyraclostrobin has a potential for bioaccumulation as BCF in fish is above the cut-off value of 500 given in the CLP Regulation.

Aquatic Toxicity

Reliable aquatic toxicity tests for both acute and chronic aquatic toxicity are available for all three trophic levels.

Acute toxicity

The summary of the relevant information on acute aquatic toxicity is provided in Table 143 of the CLH report.

For fish, seven acute toxicity studies with five different fish species were available. Rainbow trout *Oncorhynchus mykiss* was the most sensitive fish species tested in the acute studies performed according to EPA 850.1075, 72-1, with mean measured 96h LC_{50} value of 0.00616 mg/L.

Five acute toxicity studies with different taxonomic groups were provided for aquatic invertebrates. The lowest study value, according to EPA 850.1035, EPA 72-3(b), resulted in a 96h LC_{50} of 0.00416 mg/L (mean measured) for saltwater mysid *Americamysis bahia*.

Six acute toxicity studies with five different algae species were available. The freshwater diatom *Navicula pelliculosa* was the most sensitive species tested in algae acute studies performed according to EPA 123-2, EPA 850.5400, with initial measured 72h E_rC_{50} of 0.011 mg/L.

There was one toxicity study available for aquatic plants, conducted according to EPA 123-2, EPA 850.4400 and OECD 221, with mean measured 14d E_rC_{50} value of >1.077 mg/L for *Lemna gibba*.

–From the available acute aquatic toxicity data for pyraclostrobin, the DS concluded that toxicity to aquatic organisms for all three trophic levels is below 1 mg/L. Invertebrates are the most acutely sensitive taxonomic group, therefore the acute aquatic classification proposed by the DS was based on saltwater mysid *Americamysis bahia* with 96h LC_{50} of 0.00416 mg/L; experimental information for acute fish also fall within the same concentration range, supporting, thus, the classification conclusion. The DS proposed **Aquatic Acute 1** (H400) with **M-factor of 100** ($0.001 < L(E)C_{50} \leq 0.01$ mg/L).

Chronic toxicity

The summary of the relevant information on chronic toxicity is provided in Table 184 of CLP report.

There are four long-term toxicity studies with three different fish species available. Rainbow trout *Oncorhynchus mykiss* was the most sensitive fish species tested in the chronic studies performed according to OECD 210, with mean measured 98d NOEC value of 0.00235 mg/L.

Five chronic toxicity studies with different taxonomic groups were provided for aquatic invertebrates. The saltwater mysid *Americamysis bahia* was the most sensitive species tested in invertebrate chronic studies performed according to EPA 850.1000, EPA 850.1350, EPA 72-4, with a mean measured 31d NOEC of 0.000365 mg/L.

Six chronic toxicity studies with five different algae species were available. The freshwater diatom *Navicula pelliculosa* was the most sensitive species tested in algae chronic studies performed according to EPA 123-2, EPA 850.5400, with initial measured 120h NOE_rC of 0.00118 mg/L.

There was one study available for aquatic plants, conducted according to EPA 123-2, EPA 850.4400 and OECD 221, with a mean measured 14d E_rC_{10} value of 0.82 mg/L for *Lemna gibba*.

Based on the results from the long-term aquatic toxicity studies with pyraclostrobin, the DS concluded that chronic toxicity to aquatic organisms for all three trophic levels is below 0.1 mg/L. Invertebrates are the most sensitive taxonomic group. Therefore, the chronic aquatic classification proposed by DS was based on the saltwater mysid *Americamysis bahia* toxicity study with 31d NOEC of 0.000365 mg/L. The DS proposed **Aquatic Chronic 1**, with **M-factor of 100** ($0.0001 < NOEC \leq 0.001$ mg/L) along with the understanding that the substance is not rapidly degradable and has a high bioaccumulation potential.

Comments received during consultation

An MSCA, a company-manufacturer and a National Authority provided comments. The MS agreed with the proposed classification for environmental hazards by DS

Regarding aquatic toxicity, the company-manufacturer suggested some changes in endpoints and studies reliability. Comments were provided on the following acute fish studies (*Lepomis macrochirus* (14F0494/965179: 1998), *Cyprinus carpio* (11F0494/965178: 1998) and *Pimephales promelas* (18F0494/96E001: 2014)) which were considered of lower reliability by the DS, due to the fish size which was larger then recommended in the OECD TG 203.

Also, it was pointed out that bluegill study *Lepomis macrochirus* (1947-BA: 2000) conducted with smaller individuals produced essentially the same LC_{50} as the study with larger juvenile fish *Lepomis macrochirus* (14F0494/965179: 1998) that was criticized. DS pointed out that none of the studies was considered invalid or implausible because of the length deviation, and they were all considered relevant for classification purposes. However, due to the deviation from the guideline recommendation, and the associated uncertainty in sensitivity, DS retain the assessment to consider the studies reliable with restrictions.

In the view of the Company-manufacturer in case of acute toxicity study with *Americamysis bahia*, which was selected as key study for classification, the 48h LC_{50} value instead of 96h LC_{50} value should be used for classification as this is in line with Regulation (EC) 1272/2008 (section 4.1.2.7.1, Table 4.10) for crustaceans. DS responded that according to the OCSPP 850.1035 guideline, the 96h LC_{50} is the primary endpoint to be derived from acute toxicity tests on mysids. As the 48h LC_{50} and 96h LC_{50} are within the same order of magnitude, the choice of relevant endpoint does not influence the classification of the substance.

Also, it was pointed out that new and fully valid and reliable acute toxicity study with *Navicula pelliculosa* (Eckenstein, 2018) contradicts the low endpoint of the less reliable acute toxicity study with *Navicula pelliculosa* (Boeri, 2000) which was selected as key study for algae. Therefore, company-manufacturer suggested using the acute toxicity study with *Ankistrodesmus bibraianus* (Backfisch and Englert, 2018) as a key study for algae. DS pointed out that the reliability of the *N. pelliculosa* study (Boeri, 2000) was also discussed in the renewal assessment report. The RMS judged the study as valid and relevant, although some shortcomings were identified.

The company-manufacturer is of the opinion that the NOEC for F_0 male body weight in the chronic toxicity *Americamysis bahia* study (Dinehart, 2013) is of questionable value. It was proposed, instead, the NOEC of 0.00128 mg/L for F_0 reproduction as relevant endpoint in the chronic mysid study, leading to a chronic M-factor of 10. The DS highlighted that the chronic mysid study was discussed during the preparation of the renewal assessment report for pyraclostrobin and the effects on the weight of males at day 31 were considered biologically relevant, therefore the DS considered the NOEC of 0.000365 mg/L as relevant endpoint, leading to a chronic M-factor of 100.

Also, the National Authority commented on the 31-day study on *Americamysis bahia* (Dinehart, 2013) questioning the reliability of the NOEC endpoint for male weight. The DS confirmed that NOEC for *A. bahia* was used as relevant long-term invertebrate endpoint for the risk assessment.

It was asked whether it is possible to recalculate a 72-hour mean measured NOE_rC or E_rC_{10} for the study on *Navicula pelliculosa* (Boeri, 2000) considering the issues identified

regarding the analytical verification of test concentrations. The DS recalculated the effect concentrations for 72h and pointed out that E_rC_{10} of 0.000257 mg/L is extrapolated outside the tested concentration range and that the confidence intervals of the E_rC_{10} and E_rC_{20} partially overlap, most likely due to the rather flat concentration-effect relationship observed in the study; It was also mentioned that this indicates that the calculated E_rC_{10} is of low reliability and that the interpretation of the study on *N. pelliculosa* would only affect the proposed classification and M-factor if the RAC does not agree with NOEC for A*. bahia*.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS's proposal to consider pyraclostrobin as not rapidly degradable:

- The substance was stable to hydrolysis.
- The substance is not readily biodegradable. Biodegradation in the OECD TG 301 F test was 0-10% after 28 days which is below the pass level of 60 % of the test.
- In the surface water simulation test the mineralization was low (< 5% TAR) and DT_{50} were 410 to 458 days in pelagic test and 26 to 28 days for whole system in suspended solid test.
- The DT_{50} in the whole system in a water/sediment system study (System A/pond) for two labels were 23.3 days and 26.8 days (geometric mean 25.0 days). Low mineralization was observed in both test systems (max. 4.6% TAR). Three main metabolites were formed, namely BF 500-3, BF 500-6 and BF 500-7.

Bioaccumulation

RAC agrees with the DS that pyraclostrobin has a potential for bioaccumulation based on the available bioconcentration study in bluegill sunfish showing a BCF (whole fish, lipid normalized) value of 712 (chlorphenyl label) and 776 (tolyl label), which are above the CLP Regulation threshold of 500. In addition, the measured log P_{OW} of 3.99 is very close to CLP criterion of 4.

Aquatic toxicity

RAC is of the opinion that in the case of the acute toxicity study with *Americamysis bahia* (Boeri, 2000) the 96h endpoint (LC_{50}) should be used for classification because 96h exposure is in accordance with OCSPP 850.1035 guideline. The 96h exposure duration is also in line with Guidance on the Application of the CLP Criteria, section I.2.2.1 (Version 5.0, July 2017) where it is indicated that "*For other crustacea, such as mysids or others, duration of 96 hours is typical*".

In relation to the acute toxicity studies with algae, RAC is of the view that the endpoint 72h ErC50 of 0.011 mg/L from the acute toxicity study with *N. pelliculosa* (Boeri, 2000) should be used as the lowest endpoint for algae species, as the study was considered valid and relevant in both RAR and the CLH study summery although there were some shortcomings. However, RAC noted that the algae are not the most sensitive trophic level.

RAC considers the endpoint male weight from the 31-day chronic toxicity *Americamysis bahia* study (Dinehart, 2013) (NOEC of 0.000365 mg/L) a relevant endpoint for chronic hazard classification. RAC recognizes that the male weight was significantly affected by the pyraclostrobin, and the concentration-response relationship was observed at the three highest test concentrations. In addition, the NOEC based on male weight was agreed for use in risk assessment (RAR).

Acute toxicity

RAC is of the opinion that adequate acute toxicity data are available for all three trophic levels. Invertebrates are the most sensitive group and the lowest acute 96 h EC50 value of 0.00416 mg/L for *Americamysis bahia* is below the classification threshold value of 1 mg/L. RAC concludes that a **classification as Aquatic Acute 1 (H400) with an M-factor of 100** ($0.001 < EC_{50} \leq 0.01$ mg/L) is warranted.

Chronic toxicity

RAC is of the opinion that adequate chronic toxicity data are available for all three trophic levels. Invertebrates are the most sensitive group and the lowest chronic 31d NOEC value of 0.000365 mg/L for *Americamysis bahia* is below the classification threshold value of 0.1 mg/L. RAC concludes that **a classification as Aquatic Chronic 1 (H400) with an M-factor of 100** (0.0001 < NOEC ≤ 0.001 mg/L for a non-rapidly degrading substance) is warranted.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not assessed in this dossier

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

The hazard to the ozone layer was not addressed by the DS but it was open for consultation.

Comments received during consultation

One company-manufacturer pointed out that based on the very low pyraclostrobin vapour pressure (2.6 x 10-8 Pa at 20°C) and the very short half-life in air (less than 0.1 days according to Atkinson, AOPWIN program version 1.88), a risk for ozone layer depletion can be excluded.

Assessment and comparison with the classification criteria

Pyraclostrobin is not expected to remain stable in the air based on the very short half-life of less than 0.1 days, data provided in the consultation by the company-manufacturer. Due to its low half-life in the atmosphere combined with a low vapour pressure (2.6 x 10^{-8} Pa at 20°C) indicating low volatility and resulting in a low value for the Henry's Law constant (5.31 x 10^{-6} Pa m^3/mol at 25°C), pyraclostrobin is considered not to be subject to transport via air or cause hazard to ozone layer. RAC obtained the data for Henry's Law constant from the following web page http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/564.htm, available 10.6.2022.

Therefore, RAC is of the opinion that no classification is warranted for hazards to the ozone layer.

13 ADDITIONAL LABELLING

Additional labbeling with EUH070 is briefly discussed in Section 10.1.2.3.

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15 ANNEXES

Draft RAR Volume 3 B.2 (Confidential)
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