Committee for Risk Assessment
RAC

Annex 1

Background document

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

pyriproxyfen (ISO); 2-(1-methyl-2-(4-phenoxyphenoxy)ethoxy)pyridine; 4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy) propyl ether

EC Number: 429-800-1

CAS Number: 95737-68-1

CLH-O-0000007433-76-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It is based on the official CLH report submitted to consultation and additional information (if applicable).

Adopted

14 March 2024

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CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

Pyriproxyfen (ISO)

2-(1-methyl-2-(4-phenoxyphenoxy)ethoxy)pyridine; 4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propyl ether

EC Number: **429-800-1**

CAS Number: **95737-68-1**

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Version number: 2 **Date: January 2023**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PYRIPROXYFEN (ISO); 2-(1-METHYL-2-(4-PHENOXYPHENOXY)ETHOXY)PYRIDINE; 4-PHENOXYPHENYL (*RS*)-2-(2-PYRIDYLOXY) PROPYL ETHER

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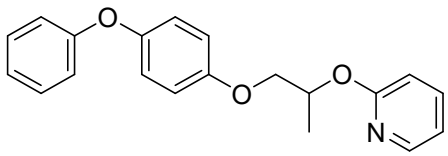
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Pyriproxyfen (ISO); 2-(1-methyl-2-(4-phenoxyphenoxy)ethoxy)pyridine; 4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propyl ether
Other names (usual name, trade name, abbreviation)	2-[1-methyl-2-(4-phenoxyphenoxy)ethoxy]pyridine
ISO common name (if available and appropriate)	Pyriproxyfen
EC number (if available and appropriate)	429-800-1
EC name (if available and appropriate)	Not applicable
CAS number (if available)	95737-68-1
Other identity code (if available)	CIPAC No. 715
Molecular formula	$C_{20}H_{19}NO_3$
Structural formula	
SMILES notation (if available)	CC(COC1=CC=C(C=C1)OC2=CC=CC=C2)OC3=CC=CC=N3
Molecular weight or molecular weight range	321.37 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	97%

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)**
2-(1-methyl-2-(4-phenoxyphenoxy)ethoxy)pyridine; 4-phenoxyphenyl (RS)-2-(2-	**97%**	**-**	Aquatic Acute 1: H400 Aquatic chronic 1: H410

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)
pyridyloxy) propyl ether			

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 (CAS no.: 108-88-3; EC no.: 203-625-9)	**0.5%**	Flam. Liq. 2: H225 Skin Irrit. 2: H315 Asp. Tox. 1: H304 STOT SE 3: H336 STOT RE 2 *: H373** Repr. 2: H361d***	Flam. Liq. 2: H225 Skin Irrit. 2: H315 Asp. Tox. 1: H304 STOT SE 3: H336 STOT RE 2: H373 Repr. 2: H361d	**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
-	-	-	-	-	-

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:

	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	613-303-00-3	pyriproxyfen (ISO); 2-(1-methyl-2-(4-phenoxyphenoxy)ethoxy)pyridine; 4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy) propyl ether	429-800-1	95737-68-1	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410			
Dossier submitters proposal	613-303-00-3	pyriproxyfen (ISO); 2-(1-methyl-2-(4-phenoxyphenoxy)ethoxy)pyridine; 4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy) propyl ether	429-800-1	95737-68-1	**Retain:** Aquatic Acute 1 Aquatic Chronic 1	**Retain:** H400 H410	**Retain:** GHS09 Wng	**Retain:** H410		**Add:** M = 10 M = 10000	
Resulting Annex VI entry if agreed by RAC and COM	613-303-00-3	pyriproxyfen (ISO); 2-(1-methyl-2-(4-phenoxyphenoxy)ethoxy)pyridine; 4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy) propyl ether	429-800-1	95737-68-1	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M = 10 M = 10000	

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

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	data conclusive but not sufficient for classification	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	hazard class not applicable	No
Pyrophoric liquids	hazard class not applicable	No
Pyrophoric solids	data conclusive but not sufficient for classification	Yes
Self-heating substances	data conclusive but not sufficient for classification	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 conclusive but not sufficient for classification	Yes
Organic peroxides	data conclusive but not sufficient for classification	Yes
Corrosive to metals	data conclusive but not sufficient for classification	Yes
Acute toxicity via oral route	data conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	data conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	data lacking	No
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	data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	data conclusive but not sufficient for classification	Yes

Hazard class	Reason for no classification	Within the scope of public consultation
Specific target organ toxicity-repeated exposure	data conclusive but not sufficient for classification	Yes
Aspiration hazard	hazard class not applicable	No
Hazardous to the aquatic environment	harmonised classification and M-Factors proposed	Yes
Hazardous to the ozone layer	data conclusive but not sufficient for classification	Yes

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Pyripoxyfen has previously been assessed for harmonised classification by TC C&L. Pyriproxyfen has a harmonised classification Aquatic Acute 1 and Aquatic Chronic 1. This has been translated from the classification decided under the Dangerous Substances Directive 67/548/EEC at the time of the European registration (DAR, Nov 2005) where it was classified as R50/53 (ATP 01).

Pyriproxyfen was notified under the Notification of New Substances (NONS) scheme of Directive 67/548/EEC, that was in place before REACH. Pyriproxyfen has one active registration under REACH (September 2019). It is noted that despite of a REACH dossier being submitted, this dossier is not updated under the REACH Regulation and thus contains very limited SID and (eco)tox information.

Furthermore, pyriproxyfen is registered as a biocidal active substance under Directive 2013/5 (https://www.echa.europa.eu/web/guest/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/61/PT18) (approval 1/2/2015 – 31/1/2025). In the assessment report for the biocidal active substance registration, classification Aquatic Acute 1 and Aquatic Chronic 1 were proposed.

Pyriproxyfen was previously approved as a plant protection product active substance in 2009. Pyriproxyfen has recently been re-evaluated for its renewal under Regulation (EC) 1107/2009. An EFSA conclusion was published in July 2019 (https://www.efsa.europa.eu/nl/efsajournal/pub/5732) and in August 2020, pyriproxyfen was renewed under Regulation (EC) 1107/2009 (Reg (EU) 2020/968).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Justification that action is needed at Community level is required.

Reason for a need for action at Community level:

Requirement for harmonised classification by other legislation or process.

Further detail on need of action at Community level

Pyriproxyfen is an active substance in the meaning of Regulation EC 1107/2009. As a result of the renewal assessment under Regulation EC 1107/2009, it is appropriate to review the existing entry in Annex VI of CLP and confirm the M-factors.

5 IDENTIFIED USES

Pyriproxyfen is an insecticide, for the control of various insect pest species in various crops. The representative uses considered for renewal of approval under Regulation EC 1107/2009 are citrus fruit and pome fruit, tomatoes (indoor and outdoor) and ornamentals (indoor and outdoor). The representative use considered for approval under Directive 98/8/EC for biocidal products was PT18 (insecticides, acaricides and products to control other arthropods).

6 DATA SOURCES

Pyriproxyfen is under evaluation for renewal of approval as a pesticide active substance according to Commission Regulation (EU) No 1107/2009. The primary sources of data are:

1. The company reports and published data contained in the renewal of approval dossier submitted by the applicant (Sumitomo Chemical Company)

2. The Renewal Assessment Report (RAR, version March 2019)

Furthermore, regarding the placing of biocidal products on the market (Dir. 98/8/EC) a CAR is available for pyriproxyfen (2012). The studies described in the CAR are also included into the pesticide renewal assessment report (RAR 2019). It is noted that the pesticide dossier (RAR 2019) contains more studies than the biocide dossier (CAR 2012).

The REACH dossier is not updated under the REACH Regulation and contains very limited information. When available, data from the REACH dossier were included in this CLH proposal.

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	Pale yellowish white solid (Technical material, Munsell colour 5Y(9/2))	Kimura, M. (2000a) NNP-0078 Kimura, M. (2000b) NNP-0079	Measured
Melting/freezing point	48.0-50.0°C	Pesselman, R.L., (1993a) NNP-31-0054	Measured
Boiling point	318°C	Isozaki, M. (2001) NNP-0086	Measured
Relative density	Not an EU data requirement under Regulation (EC) No 1107/2009	Not applicable	Not applicable
Vapour pressure	<1.33 x 10^{-5} Pa (<1.0 x 10^{-7} mmHg) at 22.81°C	Pesselman, R.L. (1989) NNP-91-0030	Measured
Surface tension	Not measured	-	Not required as the solubility of the active substance in water is below 1 mg/L.
Water solubility	At 20 °C Water solubility at pH 5: 0.058 mg/L Water solubility at pH 7: 0.101 mg/L Water solubility at pH 9: 0.119 mg/L At 25 °C Water solubility at pH 6: 0.367 mg/L	Bates, M.L. (2006) NNP-0105 ECHA dissemination site (2022)	Measured No details provided, only a value is reported

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Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Partition coefficient n-octanol/water	At 25 °C : Mean Log Pow = 4.85 at pH 5 Mean Log Pow = 4.86 at pH 7 Mean log Pow = 4.87 at pH 9 log Pow = 5.37	Bates, M. and Liney, P. (2005b) NNP-0103 ECHA dissemination site (2022)	Measured No details provided, only a value is reported
Flash point	Not measured	-	Not required as the melting point of pyriproxyfen is not below 40°C.
Flammability	Not highly flammable	Bates, M. (2001) NNP-0091	Measured
Explosive properties	Not explosive	Bates, M. (2001) NNP-0091	Theoretical assessment and DSC testing
Self-ignition temperature	No self-ignition observed up to 400 °C	Bates, M. (2001) NNP-0091	Measured
Oxidising properties	Not oxidising	Bates, M.L. (2002) NNP-0094	Theoretical assessment and DSC testing
Granulometry	Not an EU data requirement under Regulation (EC) No 1107/2009	Not applicable	Not applicable
Stability in organic solvents and identity of relevant degradation products	n-Heptane: 25 - 29 g/L at 20°C 1,2-Dichloroethane: >1000 g/L at 20°C Methanol: 25 - 29 g/L at 20°C Acetone: >1000 g/L at 20°C p-Xylene: >1000 g/L at 20°C Ethyl acetate: >1000 g/L at 20°C	Bates, M.L. (2002) NNP-0094	Measured
Dissociation constant	Dissociation constant of pyriproxyfen could not be obtained because the solubility of pyriproxyfen in water was extremely low.	Shigenaga, H. (1989) NNP-90-0022	Not measured
Viscosity	Not an EU data requirement under Regulation (EC) No 1107/2009	Not applicable	Not applicable

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 9: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
Theoretical assessment and DSC testing	Not explosive	-	Bates, M. (2001) NNP-0091

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

After theoretical assessment of the chemical structure of pyriproxyfen no explosive groups are found, the oxygen balance is less than -200 (-231.5 %). Additionally a differential scanning calorimetry (DSC) technique was applied and the exothermic decomposition energy does not have an exothermic peak >500 °C (~480 °C). Consequently it does not meet the criteria for classification as explosive. Pyriproxyfen is not explosive in the sense of Reg. (EC) No 1272/2008.

8.1.2 Comparison with the CLP criteria

The criteria for classification as an unstable explosive were not met.

8.1.3 Conclusion on classification and labelling for explosive properties

Not classified: Conclusive but not sufficient for classification.

8.2 Flammable gases (including chemically unstable gases)

Hazard class not applicable (pyriproxyfen is not a gas).

Table 10: Summary table of studies on flammable gases (including chemically unstable gases)

Method	Results	Remarks	Reference
-	-	-	-

8.2.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)

Not relevant.

8.2.2 Comparison with the CLP criteria

Not relevant.

8.2.3 Conclusion on classification and labelling for flammable gases

Not relevant.

8.3 Oxidising gases

Hazard class not applicable (pyriproxyfen is not a gas).

Table 11: Summary table of studies on oxidising gases

Method	Results	Remarks	Reference
-	-	-	-

8.3.1 Short summary and overall relevance of the provided information on oxidising gases

Not relevant.

8.3.2 Comparison with the CLP criteria

Not relevant.

8.3.3 Conclusion on classification and labelling for oxidising gases

Not relevant.

8.4 Gases under pressure

Hazard class not applicable (pyriproxyfen is not a gas).

Table 12: Summary table of studies on gases under pressure

Method	Results	Remarks	Reference
-	-	-	-

8.4.1 Short summary and overall relevance of the provided information on gases under pressure

Not relevant.

8.4.2 Comparison with the CLP criteria

Not relevant.

8.4.3 Conclusion on classification and labelling for gases under pressure

Not relevant.

8.5 Flammable liquids

Hazard class not applicable (pyriproxyfen is not a liquid).

Table 13: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
-	-	-	-

8.5.1 Short summary and overall relevance of the provided information on flammable liquids

Not relevant.

8.5.2 Comparison with the CLP criteria

Not relevant.

8.5.3 Conclusion on classification and labelling for flammable liquids

Not relevant.

8.6 Flammable solids

Table 14: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EC A.10	Not (highly) flammable	-	Bates, M. (2001) NNP-0091

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

In a standard study (EC A.10), pyriproxyfen was tested for its flammable properties. The test substance melted into a liquid which did not ignite. A few sparks of flame and a little white smoke were seen. These observations were made both in the preliminary test (train test) and the evaluation tests. Consequently it can be concluded that pyriproxyfen does not meet the criteria for classification as (highly) flammable in the sense of Reg. (EC) No 1272/2008.

8.6.2 Comparison with the CLP criteria

The criteria for classification as a (highly) flammable solid (a solid which is readily combustible, or may cause or contribute to fire through friction) were not met.

8.6.3 Conclusion on classification and labelling for flammable solids

Not classified: Conclusive but not sufficient for classification.

8.7 Self-reactive substances

Table 15: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference
-	-	-	-

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

No study conducted. The compound is not explosive, not highly flammable and not oxidising. In addition there are no chemical groups present in the molecule associated with explosive or self-reactive properties (e.g. azides, N-nitroso compounds, aromatic sulfohydrazide). Therefore the substance is not expected to be self-reactive. Consequently it does not meet the criteria for classification as self-reacting.

8.7.2 Comparison with the CLP criteria

The criteria for classification as a self-reactive substance (thermally unstable solid substances liable to undergo a strongly exothermic decomposition even without participation of oxygen (air)) were not met.

8.7.3 Conclusion on classification and labelling for self-reactive substances

Not classified: Conclusive but not sufficient for classification.

8.8 Pyrophoric liquids

Hazard class not applicable (pyriproxyfen is not a liquid).

Table 16: Summary table of studies on pyrophoric liquids

Method	Results	Remarks	Reference
-	-	-	-

8.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

Not relevant.

8.8.2 Comparison with the CLP criteria

Not relevant.

8.8.3 Conclusion on classification and labelling for pyrophoric liquids

Not relevant.

8.9 Pyrophoric solids

Table 17: Summary table of studies on pyrophoric solids

Method	Results	Remarks	Reference
-	-	-	-

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

No study conducted. Pyriproxyfen has been handled extensively in air and has never self-ignited. From the chemical structure pyriproxyfen is not expected to be pyrophoric. Consequently it does not meet the criteria for classification as a pyrophoric substance.

8.9.2 Comparison with the CLP criteria

The criteria for classification as a pyrophoric solid (solid substance or mixture which, even in small quantities, is liable to ignite within five minutes after coming into contact with air) were not met.

8.9.3 Conclusion on classification and labelling for pyrophoric solids

Not classified: Conclusive but not sufficient for classification

8.10 Self-heating substances

Table 18: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EC A.15	No auto-ignition observed below 400 °C	-	Bates, M. (2001) NNP-0091

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

In a standard study (EC A.15), pyriproxyfen did not exhibit an auto-ignition temperature up to 400 °C. In accordance with Guidance on the Application of the CLP criteria, substances or mixtures with a low melting point (< 160 °C) should not be considered for classification in this class since the melting process is endothermic and the substance-air surface is drastically reduced. The melting point of pyriproxyfen is 48 – 50 °C, therefore classification as a self-heating substance is not necessary. Consequently, it does not meet the criteria for classification as a self-heating substance.

8.10.2 Comparison with the CLP criteria

The criteria for classification as a self-heating substance (or solid substance, other than a pyrophoric solid, which, by reaction with air and without energy supply, is liable to self-heat; this substance differs from a pyrophoric solid in that it will ignite only when in large amounts (kilograms) and after long periods of time (hours or days)) were not met.

8.10.3 Conclusion on classification and labelling for self-heating substances

Not classified: Conclusive but not sufficient for classification

8.11 Substances which in contact with water emit flammable gases

Table 19: Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference
EC A.12	Does not produce flammable gases when in contact with water	-	Bates, M.L. (2002) NNP-0094

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

In a standard study (EC A.12), pyriproxyfen did not produce flammable gases when in contact with water. Additionally, based on experience in handling and use shows that the substance does not react with water to emit flammable gases. Consequently, it does not meet the criteria for classification as a substance which in contact with water will emit flammable gases in the sense of Reg. (EC) No 1272/2008.

8.11.2 Comparison with the CLP criteria

The criteria for classification as a substance which in contact with water will emit flammable gases (solid substance will interaction with water, are liable to become spontaneously flammable or to give off flammable gases in dangerous quantities) were not met.

8.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

Not classified: Conclusive but not sufficient for classification.

8.12 Oxidising liquids

Hazard class not applicable (pyriproxyfen is not a liquid).

Table 20: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
-	-	-	-

8.12.1 Short summary and overall relevance of the provided information on oxidising liquids

Not relevant.

8.12.2 Comparison with the CLP criteria

Not relevant.

8.12.3 Conclusion on classification and labelling for oxidising liquids

Not relevant.

8.13 Oxidising solids

Table 21: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
Theoretical assessment and DSC testing	Not oxidising	-	Bates, M.L. (2002) NNP-0094

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

Theoretical consideration of the chemical structure, oxygen balance and associated thermodynamic properties demonstrates that pyriproxyfen is not oxidising. Even though pyriproxyfen contains oxygen, this element is only bonded to carbon. Consequently, the classification procedure for this hazard need not be applied. Pyriproxyfen is not classified as an oxidising solid in the sense of Reg. (EC) No 1272/2008.

8.13.2 Comparison with the CLP criteria

The criteria for classification as an oxidising solid (solid substance, which, while in itself not necessarily combustible, may, generally by yielding oxygen, cause, or contribute to, the combustion of other material) were not met.

8.13.3 Conclusion on classification and labelling for oxidising solids

Not classified: Conclusive but not sufficient for classification

8.14 Organic peroxides

Table 22: Summary table of studies on organic peroxides

Method	Results	Remarks	Reference
-	-	-	-

8.14.1 Short summary and overall relevance of the provided information on organic peroxides

No study conducted, pyriproxyfen is not an organic peroxide.

8.14.2 Comparison with the CLP criteria

Pyriproxyfen is not an organic peroxide, therefore the criteria for classification as an organic peroxide (solid organic substances which contain the bivalent -O-O- structure and may be considered derivatives of H_2O_2, where one or both of the H^+ have been replaced by organic radicals) were not met.

8.14.3 Conclusion on classification and labelling for organic peroxides

Not classified: Conclusive but not sufficient for classification.

8.15 Corrosive to metals

Table 23: Summary table of studies on the hazard class corrosive to metals

Method	Results	Remarks	Reference
-	-	-	-

8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

No study conducted. The test substance is a solid which may become liquid during transport (melting point 48.0-50.0 °C) according to the wording in the UN RTDG manual. However the substance does not contain acid or basic functional groups, nor halogens, nor form complexes with metals. Consequently, testing according to the requirements of Test C.1 is not required. Pyriproxyfen is not classified as corrosive to metals in the sense of Reg. (EC) No 1272/2008.

8.15.2 Comparison with the CLP criteria

The criteria for classification as a corrosive to metals (a substance which by chemical action will materially damage, or even destroy, metals) were not met.

8.15.3 Conclusion on classification and labelling for corrosive to metals

Not classified: Conclusive but not sufficient for classification.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 24: Summary table of toxicokinetic studies

Method	Results	Reference
Metabolism	Extensively metabolised (main metabolic route is hydroxylation at the 4-position followed by a further hydroxylation step and conjugation)	CA 5.1.1/01 (1988a) CA 5.1.1/03 (1993a) CA 5.1.1/04 (1993b) CA 5.1.1/05 (1995)
Absorption	39-49% of the oral dose; overall oral absorption value estimated during active substance renewal (RAR, March 2019): 40%.	CA 5.1.1/01 (1988a) CA 5.1.1/02 (1988b) CA 5.1.1/04 (1993b)
Toxicokinetics	Whole blood: 2 mg/kg bw: C_{max} 90-400 ng/g, T_{max} 4-8 h, $T_{1/2}$ 10 h 1000 mg/kg bw: C_{max} 11-70 µg/g, T_{max} 2.8 h, $T_{1/2}$ 12 h	CA 5.1.1/02 (1988b) CA 5.1.1/04 (1993b)
Potential for accumulation	Potential for accumulation in fat ($T_{1/2}$ is 35 hours)	CA 5.1.1/01 (1988a) CA 5.1.1/02 (1988b)
Rate and extent of excretion	Rapid: mainly faecal (46-76% in 24 h) and urinary (3-9% in 24 h)	CA 5.1.1/01 (1988a)
Distribution	Widely distributed but at low levels (0.1-0.3% in tissues); highest residues in fat and liver	CA 5.1.1/02 (1988b)

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

The toxicokinetic properties of pyriproxyfen have been investigated in studies in the rat (CA 5.1.1/01, CA 5.1.1/02, CA 5.1.1/03, CA 5.1.1/04). A comparative study of metabolism in the rat and mouse has also been performed (CA 5.1.1/05., 1995).

Absorption

After single (2 or 1000 mg/kg bw) oral administration of [phenoxyphenyl-^{14}C] pyriproxyfen or [pyridyl-2,6-^{14}C] pyriproxyfen to rats absorption was approximately 39-49% in males and females, based on radiolabel recovered from urine, expired CO_2, tissues, cage wash, residual carcass and bile. Biliary excretion accounted for 34-37% of the radioactivity recovered from faeces, this is indicative of a first pass and was taken into account because the liver was identified as the target organ. In mice, absorption after a single oral dose of 2 or 1000 mg/kg bw was 35-37% of the administered radioactive dose (AR) at maximum based on the radio label recovered from urine. After repeated (2 mg/kg bw) oral administration of [phenoxyphenyl-^{14}C] pyriproxyfen, absorption was 7-14% in male and female rats, based on radiolabel recovered from urine, expired CO_2, tissues, cage wash and residual carcass. Inclusion the data on biliary excretion (34-37%) leads to a total absorbed amount of approximately 41-51%, similar to that seen after single dosing.

Elimination

Excretion of radioactivity was fast. Within 24 hours after administration, rats that had received 2 or 1000 mg/kg bw orally excreted 61-76% (M) and 46-74% (F) of the administered dose in faeces and 5-9% (M) and 3-8% (F) in urine. After repeated dosing with 2 mg/kg bw, excretion was 7-10% and 57-60% in urine and faeces, respectively. Excretion of radioactivity with expired air was small (0.1-0.4% of the administered dose). Over a 7-day period after administration faecal excretion in male and female rats was 81-93% of the applied radioactivity, while urinary excretion was 5-13%. There was no relationship with dose or dosing regimen. In mice that had received 2 mg/kg bw excretion was 10-27% and 78-90% in urine and faeces, respectively. In

mice dosed at 1000 mg/kg bw urinary and faecal excretion was 35-37% and 64-65% of the administered dose, respectively.

Distribution

Only 0.1-0.3% of the administered dose was retained in tissues (including residual carcass) of both females and males after a single oral dose of 2 or 1000 mg/kg bw and a repeated dose of 2 mg/kg bw [phenoxyphenyl-^{14}C] pyriproxyfen. In all dose groups, retention of radioactivity was highest in fat and the liver (fat low and repeated dose: 0.010-0.048 µg eq/g, high dose: 6.0-9.5 µg eq/g; liver low and repeated dose: 0.003-0.009 µg eq/g, high dose: 3.3-9.5 µg eq/g). In one of the two single dose studies in addition relatively high retention was also found in the adrenals (low dose 0.005 µg eq/g, high dose 1.3-1.5 µg eq/g) and in the kidneys of high dosed animals (2.7-3.1 µg eq/g). Radioactivity levels in tissues and organs of males were very similar to those in females. Distribution of radioactivity in tissues and organs in the single low dose group was comparable to the distribution of radioactivity in the repeated dose group.

Toxicokinetics

In single dose studies maximum residual radioactivity in tissues appeared to be 5.2-7.3% of the administered dose at 2 mg/kg bw and 1.4-2.3% at 1000 mg/kg bw. The highest C_{max} was found in the liver (low dose: 2.310-2.440 µg eq/g after 8 hours, high dose: 140-323 µg eq/g after 2-8 hours). High retention of radio activity was also found in fat, the kidney and blood (fat: low dose: C_{max} 0.280-0.461 µg eq/g after 12-24 hours, high dose: C_{max} 155-170 µg eq/g after 12-14 hours; kidney: low dose: C_{max} 0.158-0.395 µg eq/g after 4-8 hours, high dose: C_{max} 31-83 µg eq/g after 2-8 hours; blood: low dose: C_{max} 0.086-399 µg eq/g after 4-8 hours, high dose: C_{max} 11-70 µg eq/g after 2-8 hours). Half-lives were 12-17 hours for all tissues except the brain ($T_{½}$ 5-7 hours), fat ($T_{½}$ 23-35 hours) and the heart ($T_{½}$ 9-12 hours). In general half-lives for males were shorter than for females. C_{max} values were higher for males than for females with the exception of those for fat. The half-life in fat was 35 hours, which indicates a potential for accumulation.

Metabolism

Pyriproxyfen was extensively metabolised in rats of all groups. The parent compound was detected mainly in faeces (single dose 21-37%, repeated dose 6.5-11%). Minor amounts were found in urine (<2.7%). In bile the parent compound was not identified (radioactivity represented sulphate conjugates). The main metabolite in faeces was 4'-OH-pyriproxyfen, representing 23-38% and 43-54% of the total administered radioactivity in males and females, respectively. 5",4'-OH-pyriproxyfen was detected mainly in single low dose males (7.2-8.5%). In females after single and repeated dosing at 2 mg/kg bw and in high dose rats amounts of 5",4'-OH-pyriproxyfen in faeces were <2.0%. Low levels of 5"-OH-pyriproxyfen (0.1-0.3%) and DPH pyriproxyfen (0.8-1.6%) were identified after administration of [pyridyl-2,6-^{14}C] pyriproxyfen, but not after administration of [phenoxyphenyl-^{14}C] pyriproxyfen. POPA, 4-oxydiphenol, 4-oxydiphenol sulphate, 4'-OH-POPA and 4'-OH-POPA sulphate were detected in faeces only after [phenoxyphenyl-^{14}C]-pyriproxyfen. Other identified compounds in faeces were 2'-OH-pyriproxyfen, 4'-OH-pyriproxyfen sulphate, 4'-OH-pyriproxyfen glucuronide and 5",4'-OH-pyriproxyfen sulphate. None of these exceeded 5% of the administered dose. In general metabolite levels in faeces were lower in females compared to males. In urine, sulphate conjugates of 4'-oxidiphenol, 4'-OH-pyriproxyfen and 5,4'-OH-pyriproxyfen were detected in low amounts (<5%). In addition 4'-OH-pyriproxyfen (1.0-5.6%) and PYPAC (3.0-4.9%) were identified in urine of rats receiving a single dose [pyridyl-2,6-^{14}C] pyriproxyfen.

In mice receiving 2 mg/kg bw [phenoxyphenyl-^{14}C] pyriproxyfen, 4'-OH-pyriproxyfen (36-38%) was the major metabolite in faeces. At 1000 mg/kg bw, 4'-OH-pyriproxyfen was only present as 13-15% of the administered radioactivity. The parent compound accounted for 12-25% of administered radioactivity in low and high dosed mice. Low levels of 5"-OH-pyriproxyfen (0.7-2.1%) and DPH pyriproxyfen (2.6-3.1%) were identified. These levels are somewhat higher than those found in rats. In urine of high dosed mice 4'-OH-pyriproxyfen glucuronide was found to be the major metabolite accounting for 28% and 18% of the applied radioactivity in males and females, respectively. Other metabolites identified urine and faeces in mice included 2'-OH-pyriproxyfen, 4'-phenoxyphenol, POPA, 4'-oxydiphenol, 5",4'-OH-pyriproxyfen, '4' –OH-POPA and POPA sulphate. None of these exceeded 5% of the administered dose. In the bile of rats (dosed at 2 mg/kg) 4'-OH-pyriproxyfen sulphate, 4'-oxydiphenol sulphate, 4'-OH-POPA sulphate and 5",4'-OH-pyriproxyfen

sulphate were identified, but not quantified. The metabolite pattern in urine and faeces was comparable for both sexes and all dose regimes, however, the metabolite levels in faeces of females were lower. In mice, particularly at the high dose level, the metabolite levels in urine were higher than in rats. This may be indicative of a species difference.

In the blood and liver of rats dosed at 2 mg/kg bw 5",4'-OH-pyriproxyfen sulphate was the main metabolite reaching its maximum concentration 4 to 8 hours after administration (males: C_{max} 43-358 ng/g; females: C_{max} 25-37 ng/g). Concentrations of the parent were low in blood (C_{max} 8-12 ng/g after 2-24 hours) and liver (C_{max} 27-39 ng/g after 24 hours). The other metabolites identified in blood and liver were conjugated sulphates of 4'-OH-pyriproxyfen, 4'-oxydiphenol and 4'-OH-POPA. These sulphates were present at their maximum concentration in blood after 2-4 and 8 hours, males and females, respectively and in kidney after 4-8 hours for both sexes. 4'-OH-pyriproxyfen was present in minor amounts in the kidney (C_{max} 9-12 ng/g after 4 hours). In the liver highest amounts of the sulphate conjugates of 4'-OH-pyriproxyfen (C_{max} 493-770 ng/g), 4'-oxydiphenol (C_{max} 69-88 ng/g), 4'-OH-POPA (C_{max} 138-162 ng/g) and 5",4'-OH-pyriproxyfen (C_{max} 568-735 ng/g) were identified after 4-8 hours. At the same time point 4'-OH-pyriproxyfen (in males), 4'-OH-POPA and 5",4'-OH-pyriproxyfen also reached their maximum concentration in the liver, but at much levels than the sulphate conjugates. In the liver of females 4'-OH-pyriproxyfen was found in relatively high concentrations (C_{max} 337 ng/g). Pyriproxyfen was identified in small amounts in the liver 2 hours after administration (C_{max} 40-63 ng/g).

In rats pyriproxyfen is absorbed (approximately 40-50%), eliminated mainly *via* faeces (81-93%, partly after first pass metabolism) and urine (5-13%). Excretion is rapid (up to 50% within 24 hours after a single dose, up to 60% within 24 hours after repeated dose). The main metabolic route is hydroxylation at the 4-position followed by a further hydroxylation step and conjugation. Maximum radioactivity in faeces, urine, blood and tissues was found after 4-8 hours after administration (as the parent or metabolised) with the exception of fat. Metabolism in females is somewhat slower than in males. Peak levels in males are higher than in females. In mice, elimination *via* urine is higher (10-37% AR) than in rats. Metabolism in mice seems to be very similar to that in rats.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Table 25: 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 LD_{50}	Reference
OECD 401 Deviations: none	Rat, Sprague-Dawley, 5/sex	S-31183 (pyriproxyfen) 97.2% purity	1000, 2500, 5000 mg/kg bw (single dose)	>5000 mg/kg bw	CA 5.2.1/01 (1987a)
OECD 401 Deviations: none	Mouse, ICR, 5/sex	S-31183 (pyriproxyfen) 97.2% purity	1000, 2000, 5000 mg/kg bw (single dose)	>5000 mg/kg bw	CA 5.2.1/02 (1987b)
NA	Dog, beagle, 1/sex	S-31183 (pyriproxyfen) 97.2% purity	500, 1500, 5000 mg/kg bw (single dose, gelatine capsule)	NA None of the animals died, however, range finding study with only 1/sex/dose.	CA 5.2.1/03 (1986)

Table 26: Summary table of human data on acute oral toxicity

Type of data/report	Test substance,	Relevant information about the study	Observations	Reference
No human data are available				

Table 27: Summary table of other studies relevant for acute oral toxicity

Type of study/data	Test substance,	Relevant information about the study	Observations	Reference
No other relevant studies are available				

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

Studies of the acute oral toxicity of pyriproxyfen are available in the rat, mouse and dog. Studies in all three species are consistent in showing low acute oral toxicity, with acute oral LD_{50} values of >5000 mg/kg bw. Studies in the rat and mouse were performed using the deleted OECD TG 401, but are adequate for the purposes of hazard classification. The dog study is used as supporting information only.

10.1.2 Comparison with the CLP criteria

Substances are classified for acute oral toxicity in one of four following hazard categories based on (approximate) acute oral LD_{50} values from appropriate animal studies.

Category 1: LD_{50} ≤5 mg/kg bw

Category 2: LD_{50} >5 - ≤50 mg/kg bw

Category 3: LD_{50} >50 - ≤300 mg/kg bw

Category 4: LD_{50} >300 - ≤2000 mg/kg bw

The acute oral LD_{50} of pyriproxyfen was found to be >5000 mg/kg bw in the rat and mouse (Table 25). Therefore pyriproxyfen does not meet the CLP criteria for classification for acute oral toxicity.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Pyriproxyfen does not require classification for acute oral toxicity under CLP.

10.2 Acute toxicity - dermal route

Table 28: 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
OECD 402 Deviations: none	Rat, Sprague-Dawley, 5/sex	S-31183 (pyriproxyfen) 97.2% purity	2000 mg/kg bw; 24 hours (semi-occlusive)	>2000 mg/kg bw	CA 5.2.2/01 (1987c)
Comparable to OECD 402 Deviations: none	Mouse, ICR, 5/sex	S-31183 (pyriproxyfen) 97.2% purity	2000 mg/kg bw; 24 hours (semi-occlusive)	>2000 mg/kg bw	CA 5.2.2/02 (1987d)

Table 29: Summary table of human data on acute dermal toxicity

Type of data/report	Test substance,	Relevant information about the study	Observations	Reference
No human data are available				

Table 30: Summary table of other studies relevant for acute dermal toxicity

Type of study/data	Test substance,	Relevant information about the study	Observations	Reference
No other relevant studies are available				

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

Guideline-comparable studies of the acute dermal toxicity of pyriproxyfen are available in the rat and mouse. Studies in both species are consistent in showing low acute dermal toxicity, with acute dermal LD_{50} values of >2000 mg/kg bw (Table 28).

10.2.2 Comparison with the CLP criteria

Substances are classified for acute dermal toxicity in one of four following hazard categories based on (approximate) acute dermal LD_{50} values from appropriate animal studies.

Category 1: LD_{50} ≤50 mg/kg bw

Category 2: LD_{50} >50 - ≤200 mg/kg bw

Category 3: LD_{50} >200 - ≤1000 mg/kg bw

Category 4: LD_{50} >1000 - ≤2000 mg/kg bw

The acute dermal LD_{50} of pyriproxyfen was found to be >2000 mg/kg bw in a study in the rat. Therefore, pyriproxyfen does not meet the CLP criteria for classification for acute dermal toxicity. The mouse study is used as supporting information only.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Pyriproxyfen does not require classification for acute dermal toxicity under CLP.

10.3 Acute toxicity - inhalation route

Table 31: 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
OECD 403 Deviations: none	Rat, Sprague-Dawley; 5/sex	S-31183 (pyriproxyfen); 97.0% purity MMAD 0.86/0.75 µm (mist) GSD 1.35/1.55	0.6, 1.3 mg/L; 4-hour (whole body)	>1.3 mg/L; stated to be the maximum achievable concentration	CA 5.2.3/01 (1987)
OECD 403 Deviations: none	Mouse, ICR, 5/sex	S-31183 (pyriproxyfen); 97.0% purity MMAD 0.86/0.75 µm (mist) GSD 1.35/1.55	0.6, 1.3 mg/L; 4-hour (whole body)	>1.3 mg/L; stated to be the maximum achievable concentration	CA 5.2.3/02 (1987e)

Table 32: Summary table of human data on acute inhalation toxicity

Type of data/report	Test substance,	Relevant information about the study	Observations	Reference
No human data are available				

Table 33: Summary table of other studies relevant for acute inhalation toxicity

Type of study/data	Test substance,	Relevant information about the study	Observations	Reference
No other relevant studies are available				

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

Guideline-comparable studies of the acute inhalation toxicity of pyriproxyfen are available in the rat and mouse. Studies in both species are consistent in showing low acute inhalation toxicity, with acute inhalation LC_{50} values of >1.3 mg/L (no mortality was observed in either study). The highest exposure concentration used in these studies was stated to be the maximum technical achievable.

10.3.2 Comparison with the CLP criteria

Substances in the form of dusts or mists are classified for acute inhalation toxicity in one of four following hazard categories based on (approximate) acute inhalation LC_{50} values from appropriate animal studies.

Category 1: LC_{50} ≤0.05 mg/L

Category 2: LC_{50} >0.05 - ≤0.5 mg/L

Category 3: LC_{50} >0.5 - ≤1.0 mg/L

Category 4: LC_{50} >1.0 - ≤5.0 mg/L

In addition to classification for inhalation toxicity, if data are available that indicates that the mechanism of toxicity is corrosivity, the substance may also need to be labelled as EUH071 ‘Corrosive to the respiratory tract’.

The acute inhalation LC_{50} of pyriproxyfen (as a mist) was found to be >1.3 mg/L (stated to be the maximum achievable concentration) in the rat and mouse studies (Table 31). Pyriproxyfen does not therefore meet the CLP criteria for classification for acute inhalation toxicity.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Pyriproxyfen does not require classification for acute inhalation toxicity under CLP. In the absence of any significant toxicity or mortality in either study, labelling with EUH071 ‘corrosive to the respiratory tract’ is not required.

10.4 Skin corrosion/irritation

Table 34: 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
OECD 404 Deviations: occlusive dressing was used	Rabbit, NZW; 3/sex	S-31183 (pyriproxyfen) 97.2% purity	0.5 g , 4 hours (occlusive)	Mean (24-72h) scores for erythema for individual animals (0.0, 0.0, 0.0, 0.0, 0.0, 0.0) Mean (24-72h) scores for oedema for individual animals (0.0, 0.0, 0.0, 0.0, 0.0, 0.0)	CA 5.2.4/01 (1987f)

Table 35: Summary table of human data on skin corrosion/irritation

Type of data/report	Test substance,	Relevant information about the study	Observations	Reference
No human data are available				

Table 36: Summary table of other studies relevant for skin corrosion/irritation

Type of study/data	Test substance,	Relevant information about the study	Observations	Reference
No other relevant studies are available				

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

There were no signs of local effects at the application site at any time point in a guideline-compliant study performed in the rabbit.

10.4.2 Comparison with the CLP criteria

Skin corrosion is defined as the production of irreversible damage to the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to 4 hours. Corrosive reactions are typified by ulcers, bleeding, bloody scabs, and, by the end of observation at 14 days, by discolouration due to blanching of the skin, complete areas of alopecia, and scars. Histopathology shall be considered to evaluate questionable lesions. Skin irritation is defined as the production of reversible damage to the skin following the application of a test substance for up to 4 hours.

A substance is classified as corrosive to skin (Category 1) when it produces destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis in at least one tested animal after exposure for up to 4 hours. Three sub-categories are provided within the corrosion category: subcategory 1A, where corrosive responses are noted following up to 3 minutes exposure and up to 1 hour observation; sub-category 1B, where corrosive responses are described following exposure greater than 3 minutes and up to 1 hour and observations up to 14 days; and sub-category 1C, where corrosive responses occur after exposures greater than 1 hour and up to 4 hours and observations up to 14 days. Corrosive substances may be classified in Category 1 where data are not sufficient for sub-categorisation

A substance is classified as irritant to skin (Category 2) when it produces reversible damage to the skin following its application for up to 4 hours. Reversible damage is defined as:

(1) Mean score of ≥2.3-≤4,0 for erythema/eschar or for oedema in at least two of three tested animals from grading at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on three consecutive days after the onset of skin reactions;

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

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

In the absence of any local effects in the available rabbit study, pyriproxyfen does not require classification for skin corrosion or skin irritation according to the CLP criteria.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Pyriproxyfen does not require classification for skin corrosion or skin irritation under CLP.

10.5 Serious eye damage/eye irritation

Table 37: 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
OECD 405 Deviations: none	Rabbit, NZW; 3/sex	S-31183 (pyriproxyfen) 97.2% purity	0.l g (unwashed)	Mean (24-72h) scores for corneal opacity (0.0, 0.0, 0.0, 0.0, 0.0, 0.0) Mean (24-72h) scores for iris (0.0, 0.0, 0.0, 0.0, 0.0, 0.0) Mean (24-72h) scores for conjunctival erythema (0.0, 0.0, 0.33, 0.0, 0.0, 0.33) Mean (24-72h) scores for conjunctival chemosis (0.0, 0.0, 0.33, 0.0, 0.0, 0.0) All reactions had resolved within 48 hours.	CA 5.2.5/01 (1987f)

Table 38: Summary table of human data on serious eye damage/eye irritation

Type of data/report	Test substance,	Relevant information about the study	Observations	Reference
No human data are available				

Table 39: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance,	Relevant information about the study	Observations	Reference
No other relevant studies are available				

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

Minimal transient conjunctival effects were seen in a guideline-compliant study performed in the rabbit. All reactions had resolved within 48 hours.

10.5.2 Comparison with the CLP criteria

Serious eye damage (Category 1) is defined as the production of tissue damage in the eye, or serious physical decay of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application. Serious eye irritation (Category 2) is defined as the production of changes in the eye following the application of test substance to the anterior surface of the eye, which are fully reversible within 21 days of application.

A single hazard category (Category 1) is adopted for substances that have potential to seriously damage the eyes. Classification for serious eye damage (Category 1) is required for substances that produce:

(a) in at least one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or

(b) in at least 2 of 3 tested animals, a positive response of

(i) corneal opacity ≥3 and/or;

(ii) iritis >1.5

Calculated as the mean scores following grading at 24, 48 and 72 hours after instillation of the test material.

Classification for serious eye irritation (Category 2) is required for substances that produce in at least in two of three tested animals, a positive response of:

(a) corneal opacity ≥1 and/or;

(b) iritis ≥1 and/or;

(c) conjunctival erythema ≥2 and/or;

(d) conjunctival oedema (chemosis) ≥2;

Calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days

The rabbit study with pyriproxyfen showed minor transient conjunctival effects (erythema, chemosis) with no corneal or iridial effects. The reactions seen in this study are not sufficient to trigger classification for serious eye damage or for serious eye irritation.

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

Pyriproxyfen does not require classification for serious eye damage or for serious eye irritation under CLP.

10.6 Respiratory sensitisation

Table 40: 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
No animal studies are available					

Table 41: Summary table of human data on respiratory sensitisation

Type of data/report	Test substance,	Relevant information about the study	Observations	Reference
No human data are available				

Table 42: Summary table of other studies relevant for respiratory sensitisation

Type of study/data	Test substance,	Relevant information about the study	Observations	Reference
No other relevant studies are available				

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

There are no specific data on respiratory sensitisation; however the absence of marked local effects in the 28-day inhalation toxicity study with pyriproxyfen indicate an absence of respiratory sensitisation potential.

10.6.2 Comparison with the CLP criteria

A respiratory sensitiser is a substance that will lead to hypersensitivity of the airways following inhalation of the substance. Respiratory sensitisers are classed in Sub-category 1A if they show a high frequency of occurrence in humans; or a probability of occurrence of a high sensitisation rate in humans based on animal or other tests. The severity of reactions may also be considered. Respiratory sensitisers are classed in Sub-category 1B if the show a low to moderate frequency of occurrence in humans; or a probability of occurrence of a low to moderate sensitisation rate in humans based on animal or other tests (1). Severity of reaction may also be considered. Substances can be classified in Category 1 where data are not sufficient for sub-categorisation in accordance with the following criteria:

(a) if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity and/or;

(b) if there are positive results from an appropriate animal test.

The CLP Guidance also notes that, at present, recognised and validated animal models for the testing of respiratory hypersensitivity are not available. Under certain circumstances, data from animal studies may provide valuable information in a weight of evidence assessment.

There are no specific data on respiratory sensitisation; however the absence of marked local effects in the 28-day inhalation toxicity study with pyriproxyfen clearly indicates the absence of respiratory sensitisation potential. Experience from the manufacture and use of pyriproxyfen does not indicate any potential for respiratory sensitisation.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

Pyriproxyfen does not require classification for respiratory sensitisation according to the CLP criteria.

10.7 Skin sensitisation

Table 43: 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
OECD 406 (Maximisation assay) Deviations: Dose was based on article Magnussen and Kligman 1969 instead	Guinea pig (Hartley) 20 males (test group), 20 males (control group)	S-31183 (pyriproxyfen) 97.2% purity	Intradermal induction: 0.5% in corn oil Topical induction: 25% in petrolatum Topical challenge: 25% in petrolatum	No skin reactions were observed in the test or control groups at 24 or 48 hours following challenge	CA 5.2.6/01 (1987g)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
of using the highest non-irritant dose (as indicated in OECD 406)					

Table 44: Summary table of human data on skin sensitisation

Type of data/report	Test substance,	Relevant information about the study	Observations	Reference
No human data are available				

Table 45: Summary table of other studies relevant for skin sensitisation

Type of study/data	Test substance,	Relevant information about the study	Observations	Reference
No other relevant studies are available				

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

No evidence of skin sensitisation potential was seen in a guideline-compliant Maximisation study.

10.7.2 Comparison with the CLP criteria

A skin sensitiser is a substance that will lead to an allergic response following skin contact. Where data are sufficient, guidance allows the allocation of skin sensitisers into Sub-category 1A, strong sensitisers, or Sub-category 1B for other skin sensitisers. Sub-category 1A is appropriate for substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered. Sub-category 1B is appropriate for substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans. Severity of reaction may also be considered. Substances shall be classified as skin sensitisers (Category 1) where data are not sufficient for sub-categorisation in accordance with the following criteria:

(a) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons or;

(b) if there are positive results from an appropriate animal test.

Animal test results for Sub-category 1A are:

Local Lymph Node Assay: EC3 value ≤2%

Guinea pig maximisation test: ≥30 % responding at ≤0.1% intradermal induction dose or;

≥60 % responding at >0.1 - ≤1% intradermal induction dose

Animal test results for Sub-category 1B are:

Local Lymph Node Assay: EC3 value >2 %

Guinea pig maximisation test: ≥30 % to <60% responding at >0,1 % - ≤1% intradermal induction dose or;

≥30% responding at >1% intradermal induction dose

No evidence of skin sensitisation potential was seen in a guideline-compliant Maximisation study; therefore, pyriproxyfen does not meet the CLP criteria for classification as a skin sensitiser. Experience from the manufacture and use of pyriproxyfen does not indicate any potential for skin sensitisation.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Pyriproxyfen does not require classification for skin sensitisation according to the CLP criteria.

10.8 Germ cell mutagenicity

Table 46: 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	Observations	Reference
OECD 471 (1981) Deviations: none	S-31183 (pyriproxyfen) 97.2% purity	Bacterial (*Salmonella typhimurium* TA98, TA100, TA1535., TA1537, TA1538 *Escherichia coli* WP2 *uvrA*) reverse gene mutation 0, 10, 50, 100, 500, 1000, 5000 µg/plate	-/+S9: negative at up to the limit concentration	CA 5.4.1.1/01 (1998a)
OECD 473 (1983) Deviations: none	S-31183 (pyriproxyfen) 97.2% purity	Mammalian (Chinese hamster ovary) chromosome aberration 0, 10, 30, 100 µg/mL (18 & 24 hours –S9) 0, 30, 100, 300 µg/mL (2 hours +S9)	-/+S9: negative at up to the cytotoxic limit	CA 5.4.1.2/01 (1989)
OECD 476 (1984) Deviations: none	S-31183 (pyriproxyfen) 95.3% purity	Mammalian (Chinese hamster V79) forward gene mutation (*hprt* locus) 0, 10, 30, 100, 300 µg/mL (5 hours -S9) 0, 3, 10, 30, 100 µg/mL (5 hours +S9)	-/+S9: negative at up to the cytotoxic or solubility limit	CA 5.4.1.3/01 (1990)
OECD 482 (1986) Deviations: none	S-31183 (pyriproxyfen) 95.3% purity	Mammalian (HeLa S3 cells) gene mutation (unscheduled DNA synthesis) 0, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 µg/mL (3 hours -S9; Study 1) 0, 0.1, 0.2, 0.4, 0.8, 1.6 µg/mL (3 hours -S9; Study 2) 0, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6, 51.2, 102.4 µg/mL (3 hours +S9; Study 1 & 2)	-/+S9: negative	CA 5.4.1.3/02 (1988)

Table 47: Summary table of mutagenicity/genotoxicity tests in mammalian cells *in vivo*

Method, guideline, deviations if any	Test substance,	Relevant information about the study	Observations	Reference
OECD 474 (1983) Deviations: none	S-31183 (pyriproxyfen) 95.3% purity	Mouse bone marrow micronucleus CD-1 mice, 15/sex for the vehicle control; 20/sex for the pyriproxyfen group 0 and 5000 mg/kg bw (single oral gavage dose)	Negative for genotoxicity; evidence of bone marrow toxicity	CA 5.4.2/01 (1991)

Table 48: Summary table of human data relevant for germ cell mutagenicity

Type of data/report	Test substance,	Relevant information about the study	Observations	Reference
No human data are available				

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

Pyriproxyfen did not induce point mutations in *S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 and the *E. coli* strain WP2*uvrA*, both with and without metabolic activation. Pyriproxyfen was negative in a chromosome aberration test with Chinese hamster ovary cells (CHO-K1) and negative in a gene mutation test using V79 hamster cells. In addition, pyriproxyfen was negative in an *in vitro* DNA repair study using human epithelioid cells (HeLa S3). Pyriproxyfen was also shown to be negative in an *in vivo* mouse micronucleus test. In conclusion, pyriproxyfen is considered to be non-genotoxic.

10.8.2 Comparison with the CLP criteria

Mutation is defined a permanent change in the amount or structure of the genetic material in a cell. The term ‘mutation’ applies both to heritable genetic changes that may be manifested at the phenotypic level and to the underlying DNA modifications when known (including specific base pair changes and chromosomal translocations). The term ‘mutagenic’ and ‘mutagen’ are used for agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms. The more general terms ‘genotoxic’ and ‘genotoxicity’ apply to agents or processes which alter the structure, information content, or segregation of DNA, including those which cause DNA damage by interfering with normal replication processes, or which in a non-physiological manner (temporarily) alter its replication. Genotoxicity test results are usually taken as indicators for mutagenic effects. This hazard class is primarily concerned with substances that may cause mutations in the germ cells of humans that can be transmitted to the progeny. However, the results from mutagenicity or genotoxicity tests in vitro and in mammalian somatic and germ cells *in vivo* are also considered in classifying substances and mixtures within this hazard class.

For the purpose of classification for germ cell mutagenicity, substances are allocated to one of two categories. Category 1 is appropriate for substances known to induce heritable mutations (Category 1A) or substances regarded as if they induce heritable mutations in the germ cells of humans (Category 1B). Classification in Category 1A is based on positive evidence from human epidemiological studies. Classification in Category 1B is based on positive results from *in vivo* heritable germ cell mutagenicity tests in mammals, or positive results 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; or positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny. Category 2 is appropriate for substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. Classification in Category 2 is based on positive evidence obtained from somatic cell mutagenicity tests in mammals and/or in some cases from *in vitro* experiments.

No evidence for mutagenicity for pyriproxyfen was seen in a battery of studies *in vitro* and *in vivo*. Therefore, pyriproxyfen does not require classification for germ cell mutagenicity according to the CLP criteria.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Pyriproxyfen does not require classification for germ cell mutagenicity according to the CLP criteria.

10.9 Carcinogenicity

Table 49: 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
OECD 453 (1981) Rat Crl:CD® BR (SD) 50/sex/dose Deviations: clinical pathology on satellite group (10/sex/dose) at week 13, 26, 52, 78 and 104. At week 52, 10 animals/sex/satellite group were necropsied.	S-31183 (pyriproxyfen) 95.3% purity 0, 120, 600, 3000 ppm for 104 weeks, equivalent to 0/0, 5.4/7.0, 27.3/35, 138/182.7 mg/kg bw/day	No evidence of carcinogenicity. NOAEL for carcinogenicity 3000 ppm (138 and 183 mg/kg bw/d in males and females, respectively). Systemic NOAEL set at 600 ppm (27.2 mg/kg bw/day). 3000 ppm: - Decreased body weight (females: -14% week 50 and -7% week 102; males -5% week 50 and -1% week 102). - Decreased body weight gain females (-9.5% week 1-102). - Increased liver weight: absolute +27% M, +7% F (not statistically significant); relative to bw +13% M, +27% F (not statistically significant). - Increased cholesterol in M (significant in week 26 and 52 (around +46%) ; not significant in week 78 and 104. In F significant increase week 26 only (+46%). - Increased GGT in F in weeks 26 and 52; in M in week 104. - Increased ALP in M +46% week 26 (sign), +49% week 52 (sign), +65% week 78 (sign) and +11% week 104 (not sign). - Increased dark areas liver F (11/34 vs. 1/21 in control) - Increased liver necrosis in unscheduled deaths M 8/23 and F 4/16.	CA 5.5.1/01 (1991a, 1994)
OECD 451 (1981) Mouse Crl:CD-1 (ICR)BR 60/sex/dose Deviations: clinical haematology evaluation performed on 10/sex/dose in week 52 and 78.	S-31183 (pyriproxyfen) 95.3% purity 0, 120, 600, 3000 ppm (0/0, 16.4/21.1, 81.3/107.3, 422.5/532.8 mg/kg bw/day)	No evidence of carcinogenicity. Effect level for carcinogenicity >3000 ppm (422.5 and 532.8 mg/kg bw/day in males and females, respectively). An increased incidence of liver haemangiosarcoma seen in females at 3000 ppm marginally exceeds the laboratory's historical control range but is within the background range reported for this mouse strain and is not considered to be related to treatment (see 10.9.1 and 10.9.2 below for more details). A systemic LOAEL was set at 120 ppm (16.4 mg/kg bw/day) based on the reduced survival rate seen at all dose levels. 120 ppm: - Decreased survival rate: M 45% (control group 57%); , F, 56% (control group 61%). 600 ppm:	CA 5.5.1/02 (1991b, 1994)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		- Decreased survival rate (M 28%, F 45%) - Increased liver weight: interim absolute +15% F (sign); terminal not significant (absolute +7% M and F, relative +6% M) - Increased severity of systemic amyloidosis in several tissues (see table B.6.5-12 in Annex I section 3.9.1.2 for details). 3000 ppm: - Decreased survival rate (M 18%, F 36%) - Increased liver weight: F absolute interim significant (+31% absolute, +23% relative); terminal not significant (relative + 5%). - Increased severity of systemic amyloidosis in several tissues (see table B.6.5-12 in Annex I section 3.9.1.2 for details).	

Table 50: Summary table of human data on carcinogenicity

Type of data/report	Test substance,	Relevant information about the study	Observations	Reference
No human data are available				

Table 51: Summary table of other studies relevant for carcinogenicity

Type of study/data	Test substance,	Relevant information about the study	Observations	Reference
No other studies are available				

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

In a 2-year chronic toxicity and carcinogenicity study in the rat, administration of pyriproxyfen (dietary administration of 0, 120, 600 and 3000 ppm, equivalent to 0/0, 5.4/7.0, 27.3/35, 138/182.7 (m/f) mg/kg bw/day) resulted in a decrease in body weight gain at 600 and 3000 ppm. Food consumption was reduced at 3000 ppm. Changes indicative of liver toxicity (increased serum cholesterol, increased γ-glutamyl transferase, increased alkaline phosphatase) were noted in males at 3000 ppm. A decrease in globulin and increased albumin/globulin ratio was noted in females at 3000 ppm. Increased absolute and relative liver weights were noted in both sexes at 3000 ppm. At post-mortem necropsy, an increased incidence of dark areas in the liver was noted in females at 3000 ppm. Treatment-related liver necrosis was noted in males at 3000 ppm. The systemic NOAEL in this study was 600 ppm (equal to 27.3 mg/kg bw/day in males and 35 mg/kg bw/day in females), based on changes in clinical biochemistry, increased liver weights and histopathological changes in the liver at the highest dose level. There was no evidence of carcinogenicity in this study (CA 5.1.1/01), therefore, the NOAEL for carcinogenicity in this study is 3000 ppm (138 and 183 mg/kg bw/day).

In a 78-week carcinogenicity study in mice, administration of pyriproxyfen (dietary administration of 0, 120, 600 and 3000 ppm, equivalent to 0/0, 16.4/21.1, 81.3/107.3, 422.5/532.8 (m/f) mg/kg bw/day) resulted in a reduced survival rate at all dose levels. Systemic amyloidosis was identified as the primary cause of death. Most animals died in the second part of the study. A slight increased incidence in reduced motor activity and

hunched posture were noted among males and females given 3000 ppm. Mean absolute body weights were slightly reduced throughout the study period for males given 3000 ppm. A statistically significant decrease in haemoglobin was noted in females at 3000 ppm and in MCV in males at 3000 ppm. At interim sacrifice, absolute liver weight was increased in females at 600 and 3000 ppm, and relative liver weights were increased in females at 3000 ppm. In addition, at interim sacrifice, absolute spleen weights were increased in females at 3000 ppm. At terminal sacrifice relative liver weights were slightly increased at 3000 ppm in males and females. A slight increase in relative liver weight was also noted in males at 600 ppm. Absolute kidney weights were decreased in males at 3000 ppm at terminal sacrifice. At necropsy, an increased incidence in granular, pitted and/or rough kidneys was noted in males and females at 3000 ppm, mainly among the unscheduled deaths. Histopathological changes included a treatment-related increase in incidence and severity of systemic amyloidosis. Amyloidosis was noted in several organs such as the adrenal cortex, thyroid, heart, spleen, kidneys, liver, stomach, ovary, testes. An increase in severity of amyloidosis was noted in males at 600 and 3000 ppm and females at 3000 ppm, in unscheduled deaths and animals surviving until interim or terminal sacrifice. Furthermore, histopathological examination of the kidneys revealed an increased incidence of mineralization of the renal tubules in females (unscheduled deaths and terminal sacrifice), chronic progressive nephropathy in both sexes (interim and terminal sacrifice) and segmental cortical atrophy (interim and terminal sacrifice) in females at 3000 ppm. In this study a systemic LOAEL was set at 120 ppm (16.4 mg/kg bw/day) based on the reduced survival rate seen at all dose levels. During the pesticide peer review meeting (held 28 Jan – 2 Feb 2019) for the renewal of this substance, a precautionary NOAEL for carcinogenicity was set at 600 ppm, based on slightly increased incidence of liver haemangiosarcoma in females (incidences 0, 1, 1, 3 for control and increasing dose levels) even though the experts already considered this to be only an equivocal evidence for carcinogenic potential. After the meeting, the applicant provided historical control data and a statement for this finding. The historical control data indicate a range of 0 – 4.0% with a mean value of 0.8%. In the study with pyriproxyfen the incidence at the high dose level was 5% which only marginally exceeds the laboratory's historical range. Furthermore, no effect was observed in males; the findings in females did not reach statistical significance and the development of liver haemangiosarcomas was associated with animals on the border of middle age and senescence with the latency period not being decreased. RMS considers the dose level for carcinogenicity (the critical effect) in this study to be >3000 ppm (422.5 and 532.8 mg/kg bw/day).

10.9.2 Comparison with the CLP criteria

A carcinogen is defined as a substance which induces cancer or increase its incidence. Substances which have induced benign and malignant tumours in well performed experimental studies on animals are considered also to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans. For the purpose of classification for carcinogenicity, substances are allocated to one of two categories based on strength of evidence and additional considerations (weight of evidence). In certain instances, route-specific classification may be warranted, if it can be conclusively proved that no other route of exposure exhibits the hazard. A substance is classified in Category 1 for carcinogenicity (known or presumed human carcinogen) on the basis of epidemiological and/or animal data. Substances are further distinguished as Category 1A (known to have carcinogenic potential for humans), where classification is largely based on human evidence, or Category 1B (presumed to have carcinogenic potential for humans) largely based on animal studies. Classification in Category 1A and 1B is based on the strength from human studies that establish a causal relationship between human exposure to a substance and the development of cancer (Category 1A); or animal studies for which there is sufficient evidence to demonstrate carcinogenicity (Category 1B). A substances may also be classified in Category 2 (suspected human carcinogen). The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but where the evidence is not sufficiently convincing to place the substance in Category 1A or 1B.

There is no evidence for the carcinogenicity of pyriproxyfen in the rat study. In the mouse study, the incidence of liver haemangiosarcoma in females (3/60, 5%) in diets containing pyriproxyfen at 3000 ppm was marginally above the conducting laboratory's [Hazelton Laboratories America, Virginia] historical control range (0 – 4.0%), did not achieve statistical significance and was consistent with the incidence reported at other laboratories (see table, below). A low incidence of this lesion was also observed in males, however the single incidence occurred in the low dose group, was not dose related and consistent with the concurrent control. In

all cases, this lesion was associated with animals on the border of middle aged and senescence, with the lesion observed from Week 53 onwards. Overall, as there were no pre-neoplastic lesions or benign tumours in females the increase above concurrent and historical controls was marginal and restricted to a single sex. The weight of evidence suggests that in females, liver haemangiosarcoma is not related to treatment, with the incidence observed comparable with that in other laboratories and consistent with the published literature.

Haemangiosarcoma incidence in the mouse study (CA 5.5.1/02 1991b)

Parameters		♂ (ppm)				♀ (ppm)			
		0	120	600	3000	0	120	600	3000
Intake (mg/kg bw/d)		0	16.4	81.3	422.5	0	21.1	107.3	532.8
Terminal body weight (g)		36.3	35.9	38.1	35.8	30.1	29.9	30.6	28.8
Histopathology, liver									
Haemangiosarcoma	Unscheduled	1/23	0/28	0/37	0/42	0/20	1/23	0/29	2/32
	Interim	0/9	0/10	0/9	0/9	0/10	0/10	1/9	0/10
	Terminal	0/28	1/22	0/14	0/9	0/30	0/27	0/22	1/18
	Total	1/60 [1.7%]	1/60 [1.7%]	0/60 [0.0%]	0/60 [0.0%]	0/60 [0.0%]	1/60 [1.7%]	1/60 [1.7%]	3/60 [5%]
Historical data on CD-1 mice									
Hazelton Laboratories America (Virginia)		Total: 9/1148 (0.8%) Range: 0/75 - 2/50 (0-4%)							
Cohen *et al* (2009)		Mean: 3.1%							
CiToxLAB		Total: 14/712 (2.0%)				Total: 11/712 (1.4%)			
Japanese Society of Toxicological Pathology		Range 0.0-9.65 (3.2%)				Range 0.0-7.7% (1.3%)			
Charles River		*n* = 52 studies Total: 48/2941 (1.63%) Range: 1/90-6/70 (1.11-8.57%)				*n* = 54 studies Total: 25/3110 (0.80%) Range: 1/70-4/65 (1.43-6.15%)			

There is no convincing evidence in this study that pyriproxyfen causes liver haemangiosarcoma in female mice when the study data are considered in the context of the historical control incidence and the plethora of public domain data. There is insufficient evidence in the mouse oncogenicity study for a treatment-related carcinogenic effect of pyriproxyfen on the liver based on the following considerations:

- The study incidence (5%) only marginally exceeds the laboratory's limited concurrent historical control range (4%)
- A similar effect was not observed in males
- The pattern of occurrence is consistent with published data on spontaneous liver haemangiosarcoma incidences in the same strain (up to 7.7%)
- Evidence of mechanism of action attributed to chemically induced liver haemangiosarcoma is limited in the public domain, occurring in both rats and mice. No such effects were observed in the rat carcinogenicity study
- The development of liver haemangiosarcoma was exclusively associated with animals on the border of middle aged and senescence, with the latency period not being decreased and consistent with published data on spontaneous tumour incidences.

The dose level causing the critical effect (i.e. carcinogenicity) in this study is therefore >3000 ppm (422.5 and 532.8 mg/kg bw/day in males and females, respectively).

In the absence of any evidence of carcinogenicity in studies in the rat and mouse, pyriproxyfen does not meet the criteria for classification for carcinogenicity according to the CLP Regulation.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Pyriproxyfen does not require classification for carcinogenicity according to the CLP Regulation.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 52: 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
OECD 416 (1981) Rat (SD) 26/sex/dose Deviations: histopathology liver not performed on all livers of low- and mid-dose	S-31183 (pyriproxyfen) 95.3% purity 0, 200, 1000, 5000 ppm (equivalent to 0, 13.3, 66.7, 333.3 mg/kg bw/day) 10 week pre-mating exposure	No effects on fertility or reproduction; NOAEL 5000 ppm (highest dose tested). Parental toxicity: effect level 1000 ppm (66.7 mg/kg bw/day); NOAEL 200 ppm (13.3 mg/kg bw/day) 1000 ppm: - Increased relative liver weight F1 males (+10%, significant) 5000 ppm: - Reduced food consumption - Reduced body weight gain M -9% (significant) - Increased liver weight: M +12% absolute and +28% relative to bw; F +21% absolute and +27% relative to bw (all significant). - Increased relative kidney weight M (+11%, significant) - Renal pathology males (chronic interstitial nephritis 15/26 vs. 7/26 animals in the control group) Offspring toxicity: effect level of 5000 ppm (333.3 mg/kg bw/day); NOAEL set at 1000 ppm (66.7 mg/kg bw/day) 5000 ppm: - Reduced F1 pup body weight development (-16%, significant) - Reduced F2 pup body weight development (-13%, significant)	CA 5.6.1/01 (1991)
Non-guideline Rat (SD) 24/sex/dose	S-31183 (pyriproxyfen) 97.2% purity 0, 100, 300, 500, 1000 mg/kg bw/day (gavage) Males: 12 weeks (9 weeks pre-mating) Females: 2	**Parental toxicity**: Male parental toxicity effect level: 100 mg/kg bw/day based on increased adrenal weights Female parental toxicity effect level: 300 mg/kg bw/day based on clinical signs (diarrhoea), decreased body weight and increased water consumption. At the high dose level of 1000 mg/kg bw/day, two females died (on days 5 and 7 of administration). Overall, a parental LOAEL of 100 mg/kg bw/day was set. 100 mg/kg bw/day - Increased adrenal weight M absolute +8% (sign); relative +13% (sign)	CA 5.6.1/02 (1988a)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
	weeks pre-mating to GD7.	300 mg/kg bw/day: - Clinical signs F (diarrhoea 9/24) - Decreased body weight F (-4%. sign on day 7 pregnancy) - Increased water consumption M (1.3-fold compared to control, significant); F (1.2-fold, sign on day 3 and 7 of pregnancy). - Increased adrenal weight M absolute +28% (sign); relative +37% (sign) 500 mg/kg bw/day: - Clinical signs (diarrhoea 20/23 M, 22/24 F) - Decreased body weight: M -12% significant, F -5% significant - Increased water consumption M and F (1.5-2-fold, significant) - Increased absolute liver weight: M +24% (significant) - Increased adrenal weight M absolute +36% (sign); relative 56% (sign) - Decreased thymus weight M -22% (sign) 1000 mg/kg bw/day: - Mortality F (2 females died: one on day 5 and one on day 7) - Clinical signs (diarrhoea 24/24 M and F) - Decreased body weight: M -12% significant, F -9% significant - Increased water consumption (2-fold, significant) - Increased absolute liver weight M +27% (significant) - Increased absolute kidney weight M and F +8% (sign) - Increased adrenal weight: M absolute +62% (sign), relative +108% (sign); F absolute +10% (sign), relative +14% (sign) - Decreased thymus weight M -42% (sign) - Thymus atrophy M (12/24 vs. 0/24 in control group) Regarding **reproductive effects**, a lower number of corpora lutea (-10%) and live foetuses (-12%) and an increased placental weight (+10%) were observed at 1000 mg/kg bw/day. Therefore, the reproductive NOAEL was set at 500 mg/kg bw/day. No effects on **development** were observed in this study, therefore, the developmental NOAEL was set at 1000 mg/kg bw/day.	

Table 53: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	**Test substance,**	**Relevant information about the study**	**Observations**	**Reference**
No human data are available				

Table 54: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	**Test substance,**	**Relevant information about the study**	**Observations**	**Reference**
No other studies are available				

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

In a GLP- and guideline-compliant oral 2-generation reproduction study in rats performed at dietary concentrations of 0, 200, 1000 and 5000 ppm (equivalent to 0, 13.3, 66.7, 333.3 mg/kg bw/day), reduced body weight and food consumption was noted among males and females of the F_0 and F_1 generations at 5000 ppm. In addition, animals of the F_1 generation showed increased liver and kidney weights in males exposed to 1000 or 5000 ppm and increased liver weights in females exposed to 5000 ppm. Increased kidney weights in males exposed to 5000 ppm were associated microscopically with chronic interstitial nephritis. There were no changes detected between parental animals of the treated and control groups in mating indices, pregnancy rates, fertility, oestrus cycle and macroscopic findings. Examination of the F_0 and F_1 offspring revealed decreased body weights of pups in the high dose group. No treatment-related changes were detected in litter size, sex ratio, litter survival or macroscopic observations of the F_0 and F_1 offspring. Parental toxicity was apparent in this study at a dose level of 1000 ppm (equivalent to 66.7 mg/kg bw/day) and above, as shown by increased relative liver weights and increased relative kidney weights in F1 males. The effect level for offspring toxicity was 5000 ppm, equivalent to 333.3 mg/kg bw/day based on decreased body weight gain in the pups seen at this dose level. In the absence of any effects of treatment, the NOAEL for the reproductive toxicity of pyriproxyfen in this study was determined to be the highest dietary concentration of 5000 ppm, equivalent to 333.3 mg/kg bw/day (CA 5.6.1/01, 1991).

In a non-standard combined teratogenicity/reproductive toxicity study performed in rats at gavage dose levels of 0, 100, 300, 500 and 1000 mg/kg bw/day, the effect level for general male toxicity was determined to be 100 mg/kg bw/day due to increased adrenal weight (without histopathological correlates) in all treated groups. The effect level for maternal toxicity was established at 300 mg/kg bw/day; effects of treatment included clinical signs, decreased body weights, increased food and water consumption, changes in organ (liver, kidney and adrenal) weights. No effects on development were found in this study. Regarding reproductive toxicity the effect level was found to be 1000 mg/kg bw/day, as shown by decreased number of corpora lutea and live foetuses and increased placenta weights at this dose level (CA 5.6.1/02, 1988a).

	Dose (mg/kg bw/day)				
Finding	**0**	**100**	**300**	**500**	**1000**
Mortality (m/f)	0/0	0/0	0/0	1/0	0/2
Reproductive findings					
1st mating					
-copulated/mated (%)	91.7	100.0	95.8	100.0	95.5
-pregnant/copulated (%)	90.9	100.0	95.7	95.8	85.7
2nd mating					
-copulated/mated (%)	100.0	-	100.0	-	66.7(m) / 100.0(f)
-pregnant/copulated (%)	100.0	-	100.0	-	100.0
No. of dams	22	24	23	23	19
No. of corpora lutea	15.8	15.4	15.9	15.3	14.2**
Implantation rate	93.6	87.1**	95.2	91.8**	94.9
Rate of resorbed or dead foetuses	3.7	5.8	4.6	6.6	6.3
No. of live foetuses					
-total	314	302	331	301	240
-mean	14.3	12.6*	14.4	13.1	12.6*
Sex ratio	0.47	0.50	0.49	0.52	0.50
Body weight live foetuses (g)					
-male	4.82	5.11**	4.94	5.04**	5.11**
-female	4.58	4.87**	4.72*	4.76**	4.86**

Placental weight (g)					
-male	0.40	0.42	0.41	0.41	0.44**
-female	0.40	0.41	0.41	0.41	0.44**

*Statistically different from control (p≤0.05), ** Statistically different from control (p≤0.01).

10.10.3 Comparison with the CLP criteria

Reproductive toxicity includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring. For the purpose of classification the hazard class reproductive toxicity is differentiated into adverse effects on sexual function and fertility or on development; and effects on or via lactation.

Adverse effects on sexual function and fertility are defined as any effect of a substance that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.

Substances are classified in Category 1 (known or presumed human reproductive toxicant) for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans.

Classification of a substance in Category 1A (known human reproductive toxicant) is largely based on evidence from humans. Classification of a substance in this Category 1B (presumed human reproductive toxicant) is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary nonspecific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

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

No effects on fertility or reproductive capacity were observed in a guideline-compliant 2-generation reproductive toxicity study (CA 5.6.1/01, 1991) performed at dietary concentrations of pyriproxyfen of up to 5000 ppm (equivalent to 333.3 mg/kg bw/day). A non-standard study in which females were exposed to pyriproxyfen by gavage for two weeks pre-mating and up to Gestation Day 7 reports a slight reduction in the numbers of *corpora lutea* and live foetuses at the highest (and maternally toxic) dose level of 1000 mg/kg bw/day (CA 5.6.1/02, 1988a). Maternal effects at this dose level include mortality, indicating excessive toxicity. Reproductive effects are therefore considered to be secondary to toxicity and not of relevance to classification.

10.10.4 Adverse effects on development

Table 55: 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
Non-guideline Rat (SD) 24/sex/dose	S-31183 (pyriproxyfen) 97.2% purity 0, 100, 300, 500, 1000 mg/kg bw/day (gavage) Males: 12 weeks (9 weeks pre-mating) Females: 2 weeks pre-mating to GD7.	No evidence of developmental effects were seen at any dose level (effect level >1000 mg/kg bw/day) Parental toxicity: Male parental toxicity effect level: 100 mg/kg bw/d: increased liver, kidney and adrenal weights and increased water consumption Female parental toxicity effect level: 300 mg/kg bw/d based on clinical signs (diarrhoea), decreased body weight and increased water consumption. Overall, a parental LOAEL of 100 mg/kg bw/day was set. For more details, see Table 52 above (same study included for both sexual function and fertility and for developmental effects).	CA 5.6.1/02 (1988a)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Non-guideline Rat (SD) 23-24 (F)/dose	S-31183 (pyriproxyfen) 97.2% purity 0, 30, 100, 300, 500 mg/kg bw/day (gavage) GD17-LD20	Maternal toxicity effect level : 300 mg/kg bw/day as shown by mortality (3 out of 24 females), clinical signs (soft stools and diarrhoea (22/24), salivation (transient, 4/24)), reduced body weight (-9%, sign) and food consumption (-50% sign), increased water consumption (+25 to +60% sign), significantly increased liver weights (+18%), gross pathology (thymus atrophy (4/23), liver congestion (2/23) and enlargement (1/23), spleen atrophy (4/23), adrenal enlargement (5/23), kidney congestion (1/23), haemorrhage of the mucous membrane (1/23) and ulceration of the stomach (2/23)). The maternal NOAEL was set at 100 mg/kg bw/day. Developmental toxicity effect level: 100 mg/kg bw/day as shown by statistically significant changes in ambulation in males from 100 mg/kg bw/day and higher. 100 mg/kg bw/day - Increased ambulation males (+31%, sign) 300 mg/kg bw/day - Increased ambulation males (+50%, sign) - Increased dilatation renal pelvis (12 vs. 0 in control group) - Hyperaemia and/or inflammatory cell filtration propria urinary bladder (incidence of 5 vs. 0 in control, not sign) - Reduced bw live newborns: M -10% (sign), F -9% (sign) - Reduced offspring survival day 4 (-5% M, not sign) and at weaning (-4% M and -5% F, not sign) - Retarded offspring growth M up to -17% (sign); F -13% (sign) 500 mg/kg bw/day - Increased ambulation males (+37%, sign) - Increased dilatation renal pelvis (incidence of 9) - Hyperaemia and/or inflammatory cell filtration propria urinary bladder (incidence of 6, sign) - Reduced bw live newborns: M -15% (sign), F -16% (sign) - Reduced offspring survival day 4 (-12% M, not sign; -18% F, sign) and at weaning (-24% M, sign; -21% F, sign) - Retarded offspring growth M up to -20% (sign); F -20% (sign) -	CA 5.6.2/01 (1988b)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Comparable to OECD 414 (1981) Rat (SD) 36-47 (F)/dose (20-23 F sacrificed on GD 21) Deviations: none	S-31183 (pyriproxyfen) 97.2% purity 0, 100, 300, 1000 mg/kg bw/day GD 7-17	Maternal toxicity effect level: 300 mg/kg bw/day as shown by mortality,clinical signs, decreased body weight (gain), increased water consumption, increased relative liver and kidney weight. The maternal NOAEL was set at 100 mg/kg bw/day. 300 mg/kg bw/day - Mortality: 1/36 - Clinical signs (hypoactivity 1/36; wasting 1/36; hypothermia 1/36) - Decreased body weight -4% (sign) and body weight gain -20% (sign) - Increased water consumption +14 to +30% (sign) - Increased relative liver weight (+26%, not sign) and relative kidney weight (+22%, not sign). 1000 mg/kg bw/day - Mortality: 12/42 - Clinical signs (diarrhoea 42/42; erythema/swelling periproctal region 19/42; hypoactivity 10/42; wasting 9/42; rough hair 4/42; hypothermia 9/42) - Decreased body weight -12% (sign) and body weight loss during the first days of dosing (day 8-10 of gestation) - Reduced food consumption -60% (sign) - Increased water consumption +26 to +55% (sign) - Increased kidney (+7%, sign) and adrenal (+30%, sign) weights; increased relative liver weight (+11%, sign); and decreased thymus weight (-44%, sign) - Thymus atrophy (13/20), spleen atrophy (1/20), adrenal enlargement (15/20), kidney enlargement (1/20). Dead cases (12 dams) showed similar findings but also hemorrhage of the mucous membrane of the stomach (6/12). - Developmental toxicity (critical effect) level: 100 mg/kg bw/day as shown by increased numbers of foetuses with an opening of the *foramen transversarium* of the 7th cervical vertebra (see Table below in section 10.10.5 for more details). Therefore, a developmental LOAEL of 100 mg/kg bw/day was set in this study.	CA 5.6.2/02 (1988c,1989)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Comparable to OECD 414 (1981) Rabbit (Japanese White) 12-14 (F)/dose Deviations: at high dose number of dams remaining too low.	S-31183 (pyriproxyfen) 97.2% purity 0, 100, 300, 1000 mg/kg bw/day GD 6-18	Maternal toxicity effect level: 300 mg/kg bw/day. The maternal NOAEL in this study was set at 100 mg/kg bw/day. 300 mg/kg bw/day - Increased abortions or premature delivery (3/15) - Clinical signs: emaciation (3/15), lusterless fur (3/15), decrease spontaneous activity (2/15), bradypnoea (2/815). - Decreased body weight (-4%, not significant) - Decreased food consumption (-18%, not significant) 1000 mg/kg bw/day - Mortality (3/13) - Clinical signs: soft stools/diarrhoea (9/18), emaciation (10/18), lusterless fur (7/18), decreased spontaneous activity (7/18), bradypnoea (7/18) - Increased abortions or premature delivery (6/18) - Decreased body weight (-17%, significant) and body weight loss during gestation days 9 to 25. - Decreased food consumption (up to -70% compared to controls, significant) Developmental toxicity (critical effect) level: 300 mg/kg bw/day. Limited numbers of foetuses were available for assessment at 1000 mg/kg bw/day since a low number of dams remained in this top dose group. Multiple visceral malformations in 1 animals and single visceral malformation in 2 animals was observed at 300 mg/kg bw/day. See the Table below in section 10.10.5 for more details. Therefore, the developmental NOAEL was set at 100 mg/kg bw/day.	CA 5.6.2/03 (1988; 1994)
OPPTS 890.1500 (2009) Rat (SD), peripubertal 15 males/dose	S-31183 (pyriproxyfen) 99.5% purity 0, 500, 1000 mg/kg bw/day (gavage) PND23-PND53/54	At the high dose level of 1000 mg/kg bw/day lower mean body weight gains (-9%, sign) and lower mean body weights (-11%, sign) were observed. An indirect delay in the mean age at attainment of balanopreputial separation was seen at 1000 mg/kg bw/day (47.5 days compared to 45.6 days in controls). The mean body weight at the age of attainment of preputial separation was comparable to the control group (231.9 and 237.1, respectively), therefore, this delay was considered secondary to the effects on body weight gain at 1000 mg/kg bw/day. At 500 mg/kg bw/day, increased liver weight (+14% absolute; +15% relative) and hepatocellular hypertrophy (13/15 males) were observed. At 1000 mg/kg bw/day, increased liver weight (+20% absolute; +29% relative) and hepatocellular hypertrophy (14 out of 15 males) were seen; kidney weight was increased (+8% absolute; +14% relative) and kidney tubular degeneration (14 out of 15 males) and dilatation (15/15 males) was observed.	CA 5.8.3/01 (2012a)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
OPPTS 890.1450 Rat (SD), peripubertal 15 females/dose	S-31183 (pyriproxyfen) 99.5% purity 0, 500, 1000 mg/kg bw/day (gavage) PND22-PND42/43	At the high dose level of 1000 mg/kg bw/day lower mean body weight gain (-7%, sign) was observed. A delay in the mean age of attainment of vaginal opening was noted at 1000 mg/kg bw/day. This delay was considered secondary to the body weight effects in this group. There was no effect observed on age at first oestrus, oestrus cycle length, or the number of females cycling regularly. See the Table below in section 10.10.5 for more details.	CA 5.8.3/02 (2012b)

Table 56: Summary table of human data on adverse effects on development

Type of data/report	**Test substance,**	**Relevant information about the study**	**Observations**	**Reference**
No human data are available				

Table 57: Summary table of other studies relevant for developmental toxicity

Type of study/data	**Test substance,**	**Relevant information about the study**	**Observations**	**Reference**
No other studies are available				

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

In a non-standard combined teratogenicity/reproductive toxicity study performed in rats at gavage dose levels of 0, 100, 300, 500 and 1000 mg/kg bw/day, the effect level for male parental toxicity of 100 mg/kg bw/d was identified based on higher adrenal weights (without histopathological correlates). An effect level for maternal toxicity was established at 300 mg/kg bw/day. Effects of treatment included clinical signs, decreased body weights, increased food and water consumption, changes in organ weights (liver, kidneys, adrenals). Overall, the parental LOAEL was set at 100 mg/kg bw/day. In this study, no effects on development were seen, therefore the highest dose of 1000 mg/kg bw/day is considered the developmental NOAEL (CA 5.6.1/02, 1988a).

In a perinatal/postnatal toxicity study in rats performed at gavage dose levels of 0, 30, 100, 300 or 500 mg/kg bw/day, a maternal toxicity effect level of 300 mg/kg bw/day was shown by decreased body weights and food consumption, increased water consumption and increased liver weights. A developmental toxicity effect level of 100 mg/kg bw/day was shown by statistically significant changes in ambulation in males.

Table: Results for emotionality, motor coordination and learning abilities (CA 5.6.2/01, 1988b)

Parameter	**Dose (mg/kg bw/day)**				
	0	**30**	**100**	**300**	**500**
Locomotor and emotionality in open field test					
No of offspring examined, male / female	22/22	22/21	23/21	22/23	13/13
Ambulation, male / female	52.0 / 59.6	59.5 / 60.7	68.3* / 66.6	78.2** / 69.8	71.3* / 56.8
Rearing, male / female	8.3 / 9.4	9.9 / 8.5	12.4* / 10.5	9.5 / 9.2	8.7 / 6.6
Preening, male / female	1.5 / 0.8	0.7* / 0.7	1.0 / 1.0	0.5* / 0.6	0.6 / 1.2
Defecation, male / female	1.5 / 0.5	1.0 / 0.2	0.5* / 0.7	1.0 / 0.2	0.5 / 0.6

Motor coordination by rotarod					
Median of falls, male / female	2.0 / 4.0	2.5 / 4.0	4.0 / 4.0	3.0 / 4.0	2.0 / 6.0
Learning ability in T-maze test[a]					
Time 1st day, male/female	70.1 / 50.3	66.4 / 48.6	75.7 / 52.1	66.2 / 51.3	69.0 / 56.1
Time 2nd day, male/female	254.3 / 180.9	222.6 / 186.7	236.0 / 217.1*	214.5* / 199.6	231.5 / 199.5
Time 3rd day, male/female	108.1 / 76.9	101.9 / 88.5	107.5 / 76.0	93.6 / 83.3	106.7 / 81.3

[a]Time during 1st day represent harmonic mean of total elapsed time (sec) during 5 trials in a straight channel; time during 2nd and 3rd day represents harmonic mean of total elapsed time (sec) during 5 trials in a maze.

*significantly different from control ($p \leq 0.05$), **significantly different from control ($p \leq 0.01$).

Increased numbers of stillborn pups were apparent at the highest (maternally toxic) dose level only. Significantly reduced body weights of live newborns were observed at 300 and 500 mg/kg bw/day and significantly reduced offspring survival rates on day 4 and at weaning at 500 mg/kg bw/day. No abnormalities were detected in the development of pups following morphological examinations and functional, sensory, learning and reproductive ability tests. Assessment of offspring motor activity in the open field test in this study (1/sex/litter, at approximately 4 weeks of age) showed significantly higher levels of ambulation in male offspring at 100, 300 and 500 mg/kg bw/d, when compared to the concurrent control value. In this study a developmental NOAEL of 30 mg/kg bw/day was set based on the changes in ambulation in male pups (CA 5.6.2/01, 1988b).

A developmental toxicity study in the rat was performed at dose levels of 0, 100, 300 or 1000 mg/kg bw/day; concurrent groups of dams were similarly treated and were allowed to deliver naturally. The effect level for maternal toxicity in this study was found to be 300 mg/kg bw/day, as shown by mortality, clinical signs, decreased body weights and food consumption, increased water consumption, macroscopic findings and associated organ weight changes. A developmental toxicity effect level of 100 mg/kg bw/day was identified for this study, as shown by increased incidences of foetal skeletal variations, specifically opening of the *foramen transversarium* of the 7th cervical vertebra.

Table: Skeletal findings (variations) in foetuses (CA 5.6.2/02, 1988c 1989)

Parameters	Dose level (mg/kg bw/day)			
	0	100	300	1000
Examination of foetuses				
- No. of foetuses examined (litters)	202 (23)	200(23)	200 (23)	154 (18)[a]
Skeletal findings (variation)				
No of foetuses with skeletal variation (%)	14 (6.9)	14 (7.0)	15 (7.5)	37 (24.0)**
-Cervical rib	6	3	2	1
-Lumbar rib	7	6	4	11
-Shortening of 13th rib	1	1	1	1
-7 lumbar vertebrae	1	1	0	1
-Opening of foramen transversarium of the 7th cervical vertebrae	0	3	10*	22**

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

[a]Two dams in the high dose group had total litter resorption, therefore results from 18 litters are given.

Among the dams that were allowed to litter, no abnormalities were found in the number of females with live newborns, parturition, gestation period, numbers of implantations, liveborn/stillborn pups, pup weights and viability. There were no morphological changes observed in foetuses or pups that were attributed to treatment. Furthermore no abnormalities were detected in the development of pups following functional, sensory, learning and reproductive ability tests. In this study a developmental LOAEL of 100 mg/kg bw/day was set based on the increased incidence of opening of the foramen transversarium of the 7th cervical vertebra seen at all dose levels tested (CA 5.6.2/02, 1988c 1989).

In a developmental study in the rabbit performed at gavage doses of 0, 100, 300 and 1000 mg/kg bw/day, an effect level for maternal toxicity of 300 mg/kg bw/day was identified on the basis of mortality, increased abortion and/or premature delivery, clinical signs, decreased body weight, decreased food consumption and macroscopic findings. The number of surviving dams in the top dose group was insufficient for useful evaluations. At 300 mg/kg bw/day, multiple visceral malformation were seen in one animal and single visceral malformation in two animals.

Table: Skeletal and visceral findings (CA 5.6.2/03, 1988, 1994)

Parameter	Dose (mg/kg bw/day)			
	0	100	300	1000
Skeletal examination				
No. of examined foetuses	93	90	89	26
Defect 3rd distal phalanx hinder leg	0	0	1 (1.1)	0
Fusion cervical vertebrae	12 (12.9)	9 (10.0)	0 (0.0)	2 (7.7)
Assymetric sternebrae	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Hypoplasia 3rd distal phalanx foreleg	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Hypoplasia 2nd distal phalanx hinder leg	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
13 ribs	7 (7.5)	3 (3.3)	5 (5.6)	2 (7.7)
No. of ossified middle phalanges	3.5	3.8*	3.7	3.8
Visceral examination				
No. of foetuses examined	93	90	89	26
Cystic lung	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Hypoplasia left atrial auricle	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Persistent truncus arterious	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Ventricular septal defect	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Defect gallbladder	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Persistent left azygos vein	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Abnormal location posterior vena cava	17 (18.3)	14 (15.6)	14 (15.7)	9 (34.6)
Abnormal location right subclavian artery	5 (5.4)	0 (0.0)	5 (5.6)	0 (0.0)
Bifurcation vermiform appendix	0 (0.0)	2 (2.2)	2 (2.2)	1 (3.8)

Numbers in table are given as: no. of foetuses with the malformation (incidence).
*statistically different from control ($p \leq 0.05$).

Considering this finding and the fact that the high dose of 1000 mg/kg bw/day could not be assessed as an insufficient number of dams remained at this high dose, a developmental NOAEL of 100 mg/kg bw/day was set (CA 5.6.2/03, 1988, 1994).

A pubertal development assay was performed in intact juvenile/peripubertal male rats given dose levels of 0. 500 or 1000 mg/kg bw/day by gavage from postnatal day 23 to postnatal day 53/54. In this study, a non-significant delay in complete balanopreputial separation was seen at 1000 mg/kg bw/day. However, this seems to be an indirect effect related to lower body weight gain in this group compared to the control.

Table: Summary of balanopreputial separation from the *in vivo* intact juvenile/peripubertalassay in male rats (CA 5.8.3/01, 2012a)

Parameter	♂ (mg/kg bw/d)		
	0	500	1000
Mean ±SD age at PPS (days)			
PND ANOVA (PND) ANCOVA (PND)	45.6 ±2.56 45.6 45.6	46.5 ±1.73 46.6$^{NS, -}$ 46.6 $^{NS,-}$	47.5 ±3.50 47.4^{NS,NS} 47.4 ^{NS,NS}
Mean ±SD age at PPS (incomplete)			
PND ANOVA (PND) ANCOVA (PND)	42.9 ±1.94 43.0 43.0	43.9 ±2.34 43.9$^{NS,-}$ 43.9$^{NS,-}$	44.5 ±1.77 44.5^{NS,*} 44.5^{NS,*}

Parameter	♂ (mg/kg bw/d)		
	0	500	1000

PND: post-natal day
PPS: balanopreputial separation
ANOVA: analysis of variance
ANCOVA: analysis of covariance
Subscript values refer to statistical analysis conducted by the Dunnett's test and Trend test analysis
NS: not significant
* $p \leq 0.05$

Systemic toxicity was seen in this study: decreased body weight and body weight gain at 1000 mg/kg bw/day, increased liver weight and hepatocellular hypertrophy at 500 and 1000 mg/kg bw/day, increased kidney weight and kidney tubular degeneration and dilatation at 1000 mg/kg bw/day (CA 5.8.3/01, 2012a).

A pubertal developmental assay was performed in intact juvenile/peripubertal female rats given dose levels of 0, 500 or 1000 mg/kg bw/day by gavage from postnatal day 22 to postnatal day 42/43. In this study, a delay in the mean age of attainment of vaginal opening was noted at 1000 mg/kg bw/day. This delay was considered secondary to the body weight effects noted in this group. No effects on age at first oestrus or oestrus cyclicity were observed.

Table: Summary of vaginal opening and oestrus cycling data the *in vivo* intact juvenile/peripubertal assay in female rats (CA 5.8.3/02, 2012b)

Parameter	♀ (mg/kg bw/d)		
	0	500	1000
Vaginal opening			
- Age (PND) (u/a): - Age (PND) incomplete (u/a): - Body weight at VO (g) (u/a):	36.3 / 34.7 33.7 / 32.6 141.6 / 131.1	35.3 / 33.8 34.9 / 33.8 131.1 / 121.5	37.1 / 36.0 36.1* / 35.2* 130.8 / 123.7
Oestrous cyclicity			
- Mean age at 1st vaginal oestrous (PND): - Mean cycle length (days): - % cycling: - % regularly cycling:	37.7 4.7 100 83.3	37.0 4.7 100 70	38.4 3.8 92.9 60

* $p \leq 0.05$
PND: post-natal day
VO: vaginal opening
u/a: unadjusted / adjusted

Systemic toxicity was seen in this study: decreased body weight and body weight gain at 1000 mg/kg bw/day, increased liver weight and hepatocellular hypertrophy at 500 and 1000 mg/kg bw/day, increased kidney weight and kidney tubular degeneration and dilatation at 1000 mg/kg bw/day (CA 5.8.3/02, 2012b).

10.10.6 Comparison with the CLP criteria

Reproductive toxicity includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring. For the purpose of classification the hazard class reproductive toxicity is differentiated into adverse effects on sexual function and fertility or on development; and effects on or via lactation.

Adverse effects on development of the offspring (developmental toxicity) includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatal, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include death of the developing organism, structural abnormality, altered growth, and functional deficiency.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PYRIPROXYFEN (ISO); 2-(1-METHYL-2-(4-PHENOXYPHENOXY)ETHOXY)PYRIDINE; 4-PHENOXYPHENYL (*RS*)-2-(2-PYRIDYLOXY) PROPYL ETHER

Substances are classified in Category 1 (known or presumed human reproductive toxicant) for reproductive toxicity when they are known to have produced an adverse effect on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans.

Classification of a substance in Category 1A (known human reproductive toxicant) is largely based on evidence from humans. Classification of a substance in this Category 1B (presumed human reproductive toxicant) is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary nonspecific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

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

In the standard rat developmental study (CA 5.6.2/02, 1988c), a statistically significant increase in the proportion of foetuses with skeletal variations was seen at the highest dose level of 1000 mg/kg bw/day, as shown in the table below.

Summary of relevant findings (CA 5.6.2/02, 1988c)

	Dose level (mg/kg bw/d)			
	0	**100**	**300**	**1000**
Dams examined (#)	36	36	36	42
Maternal mortality	-	-	1	12
Maternal clinical signs:				
Diarrhoea	-	-	-	42
Periproctal erythema / swelling	-	-	-	19
Hypoactivity	-	-	1	10
Wasting	-	-	1	9
Rough hair	-	-	-	4
Lacrimation	-	-	-	2
Hypothermia	-	-	1	9
Blanching (auricle and extremity)	-	-	1	3
Nasal staining	-	-	-	6
Maternal bodyweight (g): GD17	319.8	314.1	307.7*	288.6**
Maternal bodyweight (g): GD21	370.1	366.6	357.7	341.0**
Foetuses (litters) examined	202 (23)	200(23)	200 (23)	154 (18)
Foetuses with skeletal variations (%)	14 (6.9)	14 (7.0)	15 (7.5)	37 (24.0)**
Opening of foramen transversarium (7^{th} cervical vertebra)	0 -	3 1.5%	10* 5.0%	22** 14.3%

**significantly different to controls ($p<0.05$); **$p<0.01$*

Historical control data relevant to the study of CA 5.6.2/02, 1988c

Year	**No. studies**	**No. foetuses**	**Opening *foramen transversarium* of the 7th cervical vertebra**			
			Total no.	**Min - Max**	**Mean (%)**	**Min-Max (%)**
1984	8	1422	25	0-6	1.76%	0.0-3.2%
1985	6	1143	12	0-4	1.05%	0.0-2.5%
1986	7	1141	12	0-3	0.85%	0.0-1.7%
1984-1986	21	3706	49	0-6	-	0-3.2%

Data from Report NNT-41-0124: 1984-1986

Findings at 1000 mg/kg bw/day in this study are attributable to a significant increase in the incidence of a single variation; namely opening of *foramen transversarium* of the 7th cervical vertebra. The incidence of this finding was also significantly increased at 300 mg/kg bw/day and was marginally (but not significantly) increased compared to the concurrent control group at 100 mg/kg bw/day. Based on this finding, a developmental LOAEL was set at 100 mg/kg bw/day during the pesticide peer review meeting (PPR 190, Jan-Feb 2019). This finding is associated with maternal toxicity in all dose groups, including a high level of mortality at 1000 mg/kg bw/day. Signs of toxicity and significantly reduced weight gain were also apparent in dams at 300 mg/kg bw/day; reduced weight gain was also apparent in dams at 100 mg/kg bw/day. The high dose level of 1000 mg/kg bw/day was therefore clearly excessive; the findings at this dose level are therefore not of relevance for classification. The findings at 300 mg/kg bw/day are associated with reduced maternal body weight gain and clinical signs and are therefore of less relevance to classification. Historical control data from the testing laboratory for this strain of rat (21 studies performed between 1984 and 1986) report a background incidence for this specific finding of 0-3.2%. The incidence of this finding (1.5%) reported in this study at the low dose level 100 mg/kg bw/day is clearly within the historical range. The incidences at 300 and 1000 mg/kg bw/day exceed the historical range, but are associated with maternal toxicity. Maternal toxicity at 1000 mg/kg bw/day was marked and excessive; maternal toxicity was less marked at 300 mg/kg bw/day but was still evident from the clinical signs (i.e. hypoactivity, wasting, hypothermia) and statistically significant effects on bodyweight (up to 21% less body weight gain compared to control). Taking into account the relevant historical control data, the increased incidence of the specific finding (opening of foramen transversarium (7th cervical vertebra)) in this study at 100 mg/kg bw/day is considered to be incidental. The increased incidence of this single finding (variation) at 300 and 1000 mg/kg bw/day is likely to be related to treatment with pyriproxyfen but is not of relevance to classification due to the association with maternal toxicity. It is also notable that other foetal skeletal parameters were unaffected by treatment with pyriproxyfen. Overall, this finding is not considered for classification for developmental toxicity(CA 5.6.2/02, 1988c).

In the standard developmental rabbit study (CA 5.6.2/03, 1988) groups of 12-14 pregnant JW-NIBS rabbits were gavaged with pyriproxyfen at dose levels of 0, 100, 300 or 1000 mg/kg bw/day on Gestation Days 6-18. Dams were terminated on Gestation Day 28. Higher incidences of abortion were reported at 300 mg/kg bw/day (three dams) and 1000 mg/kg bw/day (six dams), compared to a single incidence in the control group. Three deaths occurred in dams at the high dose level. Signs of toxicity were reported at 1000 mg/kg bw/day (soft/mucous stool, diarrhoea, inability to stand, emaciation, dull fur, hypoactivity, bradypnoea) and at 300 mg/kg bw/day (soft stool, emaciation, dull fur, hypoactivity, bradypnoea). Mean maternal bodyweights were significantly lower than controls from Gestation Day 12-25 at 1000 mg/kg bw/day due to weight loss during the treatment phase. Initial weight loss was also seen at 300 mg/kg bw/day. Weight gain over the treatment phase was reduced in this group; however mean bodyweight values were not significantly different to controls. Food consumption was significantly reduced at 1000 mg/kg bw/day from Gestation Days 9-22, and was lower (although not significantly) at 300 mg/kg bw/day. Due to the high incidences of mortality and abortion, the number of litters available for assessment at the highest dose level of 1000 mg/kg bw/day was limited. One foetus at 300 mg/kg bw/day (Dam #306) showed multiple visceral malformations (cystic lung, hypoplasia of the left atrial auricle, persistent truncus arteriosus and ventricular septal defect). The other five of the six foetuses in this litter did not show any visceral malformations. One foetus from another litter in the 300 mg/kg bw/day dose group (Dam #310) also showed a gallbladder defect; the other eight of the nine foetuses in this litter did not show any malformations. Persistent left azygous vein (an anomaly) was reported for one foetus of this group (Dam #312). The incidence of foetal visceral variations showed an even distribution across the dose groups.

Incidence of foetal visceral findings (CA 5.6.2/03, 1988)

		Dose level (mg/kg bw/d)			
		0	**100**	**300**	**1000**
Litters (#)		13	12	11	4
Foetuses examined (#)		93	90	89	26
Malformations	Cystic lung	0 (0.0)	0 (0.0)	1[a] (1.1)	0 (0.0)
	Hypoplasia left atrial auricle	0 (0.0)	0 (0.0)	1[a] (1.1)	0 (0.0)
	Persistent truncus arteriosus	0 (0.0)	0 (0.0)	1[a] (1.1)	0 (0.0)
	Ventricular septal defect	0 (0.0)	0 (0.0)	1[a] (1.1)	0 (0.0)
	Defect gallbladder	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Anomaly	Persistent left azygous vein	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Variations	Abnormal location posterior vena cava	17 (18.3)	14 (15.6)	14 (15.7)	9 (34.6)
	Abnormal location right subclavian artery	5 (5.4)	0 (0.0)	5 (5.6)	0 (0.0)
	Bifurcation vermiform appendix	0 (0.0)	2 (2.2)	2 (2.2)	1 (3.8)

Foetal incidence (% incidence)
[a]*one foetus with multiple findings*

Due to the low number of litters at 1000 mg/kg bw/day, this dose level was considered to be insufficient for useful foetal evaluations. While the incidence of foetal visceral malformations at 300 mg/kg bw/day is elevated compared to the concurrent controls, this finding is largely attributable to a single foetus with multiple malformations. The total incidence in this group of foetuses with visceral malformations (2/89; 2.2%) is within the historical control range of 0.0-3.3% (studies performed between 1982-1988); one study in the historical control range reports three foetuses (3.3%) with visceral malformations. Furthermore, all malformations in the current study are reported with a single incidence, do not attain statistical significance and are known to occur spontaneously. While the low number of litters available for assessment at the high dose level is noted, the complete absence of foetal visceral malformations in this group is notable. During the pesticide peer review meeting (PPR 190, Jan-Feb 2019), it was decided to set the developmental NOAEL at 100 mg/kg bw/day based on multiple visceral malformations in 1 animal and single visceral malformations in 2 animals at 300 mg/kg bw/day and the fact that the high dose of 1000 mg/kg bw/day could not be assessed due to an insufficient number of dams remaining. However, considering that the incidence of visceral malformations is within historical control data and that the reported malformations are found with a single incidence, it is concluded that these are not relevant for the purpose of classification.

A non-standard rat study in which females were exposed to pyriproxyfen by gavage for two weeks pre-mating and up to Gestation Day 7 reports a reduction in the numbers of *corpora lutea* and live foetuses at the highest dose level of 1000 mg/kg bw/day which is considered a reproductive effect (CA 5.6.1/02, 1988a). In this study no developmental effects were observed up to the highest dose level tested, therefore, no effects relevant for classification for developmental toxicity were observed.

In a further non-standard study (CA 5.6.2/01, 1988b), rats were administered pyriproxyfen by gavage at dose levels of up to 500 mg/kg bw/day from Gestation Day 17 to Lactation Day 20. Foetal weights were significantly reduced at birth; increased incidence of dilatation of the renal pelvis, hyperaemia and/or inflammatory cell infiltration in the propria of the urinary bladder was noted in the 300 and 500 mg/kg bw/day dose groups. A statistically significant increase in ambulation was seen in male pups at 100, 300 and 500 mg/kg bw/day compared to the control group. During the pesticide peer review meeting (PPR 190, held Jan-Feb 2019) this effect was taken into account for developmental NOAEL setting. However, considering there is no dose-response relationship and in the absence of correlating findings or similar effects in female offspring, this increase in ambulation in male pups is not considered relevant for classification for developmental toxicity.

In a pubertal development study, male rats were exposed to 0, 500 or 1000 mg/kg bw/day from postnatal day 23 to postnatal day 53/54 (CA 5.8.3/01, 2012a). At the high dose level of 1000 mg/kg bw/day, lower body

weight and body weight gain were observed. An indirect delay in the mean age of attainment of balanopreputial separation was observed at 1000 mg/kg bw/day. The mean body weight at the age of attainment of preputial separation was comparable to the control group, therefore, this delay was considered secondary to the effects on body weight gain at 1000 mg/kg bw/day. Therefore, this delay in preputial separation is not considered for classification for a developmental effect.

In a pubertal development study, female rats were exposed to 0, 500 or 1000 mg/kg bw/day from postnatal day 22 to postnatal day 42/43 (CA 5.8.3/02, 2012b). At the high dose level of 1000 mg/kg bw/day lower mean body weight gain was observed. A delay in the mean age of attainment of vaginal opening was noted at 1000 mg/kg bw/day. This delay was considered secondary to the body weight effects in this group. There was no effect observed on age at first oestrus, oestrus cycle length, or the number of females cycling regularly. Therefore, this delay in vaginal opening is not considered for classification for a developmental effect.

10.10.7 Adverse effects on or via lactation

Table 58: 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
No additional study was carried out.			

Table 59: Summary table of human data on effects on or via lactation

Type of data/report	Test substance,	Relevant information about the study	Observations	Reference
No human data are available				

Table 60: Summary table of other studies relevant for effects on or via lactation

Type of study/data	Test substance,	Relevant information about the study	Observations	Reference
No other studies are available				

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

No additional study was carried out.

10.10.9 Comparison with the CLP criteria

Adverse effects on or via lactation are included under reproductive toxicity, but for classification purposes such effects are treated separately. This is because it is desirable to be able to classify substances specifically for an adverse effect on lactation so that a specific hazard warning about this effect can be provided for lactating mothers.

According to Regulation EC No 1272/2008 (CLP), Table 3.7.2.2.2, classification for lactation effects is 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.

In the 2-generation study in rats, pup body weight development was decreased in F1 and F2 pups during lactation at 5000 ppm (high dose group). The effect was observed during the last week of lactation, when pups already start eating diet. Therefore, the pups can be exposed via the diet during this last stage of the study. In addition, maternal toxicity was observed at 5000 ppm. The effect on pup body weight during the last week of lactation is not considered to be sufficient for classification and labelling for lactation effects.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Pyriproxyfen does not meet the CLP criteria for classification for reproductive toxicity.

10.11 Specific target organ toxicity-single exposure

Table 61: 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
No method Rat (SD) 3/sex Range finding acute neurotoxicity study	Pyriproxyfen T.G. 99.5% purity 0, 300, 1000, 2000 mg/kg bw (single gavage dose)	Effect level >2000 mg/kg bw. Under the conditions of this study 2000 mg/kg bw was tolerated and considered to be a suitable maximum dose for the definitive acute oral neurotoxicity study. The time of peak effect was determined to be 8 hours post dosing.	CA 5.7.1/01 (2010)
OECD 424 (1997) Rat (SD) 12/sex Acute neurotoxicity study	Pyriproxyfen T.G. 99.5% purity 0, 300, 1000, 2000 mg/kg bw (single gavage dose)	Unkempt appearance was noted in the 2000 mg/kg bw group males (1/12) and females (6/12) at the detailed physical examination approximately 24 hours following dose administration. Therefore, the NOAEL for general toxicity is set at 1000 mg/kg bw. In males a decrease in total and ambulatory motor activity counts was observed at 1000 and 2000 mg/kg bw. See the Table below in section 10.11.2 for details. Therefore, the NOAEL for neurotoxicity is 300 mg/kg bw.	CA 5.7.1/02 (2011a)
OECD 401 Deviations: none Rat (SD) 5/sex/ dose	Pyriproxyfen (S-31183), 97.2% purity 1000, 2500, 5000 mg/kg bw (single dose via gavage)	No mortality 2500 mg/kg bw - Decreased motor activity in males (1 animal at 4 hr after administration). 5000 mg/kg bw	CA 5.2.1/01 (1987a)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		- Decreased motor activity day 0 and 1 (M: 2 males 2hrs, 3 males 4hrs, 4 males day 1; F 1 female 2hrs, 1 female 4hrs, 2 females day 1). - Soft faeces and diarrhoea day 0 and 1 (M: 2 males 4hrs, 5 males day 1; F 2 females 4hrs, 4 females day 1). - Decreased body weight (-7%, sign) and body weight gain (-32%, sign) in males at day 7; decreased body weight in females at day 7 (-9%, sign) and day 14 (-7%, sign) and decreased body weight gain in females on day 7 (-35%, sign) and day 14 (-22%, sign).	
OECD 401 Deviations: none Mouse, ICR 5/sex/dose	Pyriproxyfen (S-31183) 97.2% purity 1000, 2000, 5000 mg/kg bw (single dose via gavage)	2000 mg/kg bw - Mortality: 2/5 males within 2 days after treatment. - Ataxic gait: one male 1 day after treatment - Irregular respiration: 1 male one day after treatment 5000 mg/kg bw - Mortality: 2/5 males within 2 days after treatment; 1/5 females on day 1. - Ataxic gait and irregular respiration: 3 males one day after treatment and 1 male two days after treatment; 1 female one day after treatment and 1 female 2 days after treatment. - Decreased spontaneous activity: 1 male three days after treatment and 1 male four days after treatment; 1 female three days after treatment and 1 female four days after treatment. - Decreased body weight gain: males -30% on day 7, returned to control values on day 14.	CA 5.2.1/02 (1987b)
NA Dog, beagle 1/sex/dose (range finding study)	Pyriproxyfen (S-31183) 97.2% purity 500, 1500, 5000 mg/kg bw (single oral dose via capsule)	No mortality No effects on food consumption, body weight, haematology, blood biochemistry, or pathology at any dose level. 5000 mg/kg bw: - Vomiting: 1 male and 1 female at day 1.	Ca 5.2.1/03 (1986)
OECD 402 Deviations: none Rat (SD) 5/sex	Pyriproxyfen (S-31183) 97.2% purity 2000 mg/kg bw, dermal (24h semi-occlusive)	No mortality No clinical signs, effects on body weight or pathology.	CA 5.2.2/01 (1987c)
Comparable to OECD 402 Deviations: none	Pyriproxyfen (S-31183) 97.2% purity 2000 mg/kg bw, dermal	No mortality No clinical signs, effects on body weight or pathology.	CA 5.2.2/02 (1987d)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Mouse, ICR 5/sex	(24h semi-occlusive)		
OECD 403 Deviations: none Rat (SD) 5/sex/dose	Pyriproxyfen (S-31183) 97.2% purity 0.6 or 1.3 mg/L, inhalation (4hr, whole body)	No mortality No effects observed at 0.6 mg/L. No treatment-related effects on pathology or histopathology were observed at the two dose levels tested. 1.3 mg/L: - Salivation: 2/5 males and 1/5 females observed at the 4th hour of exposure. - Urinary incontinence: 2/5 females, observed at the 4th hour of exposure - Decreased body weight gain in males on day 3 (-18%, sign); no difference on day 7 or day 14.	CA 5.2.3/01 (1987)
OECD 403 Deviations: none Mouse, ICR 5/sex/dose	Pyriproxyfen (S-31183) 97.2% purity 0.6 or 1.3 mg/L, inhalation (4hr, whole body)	No mortality. No treatment-related effects on pathology or histopathology. 1.3 mg/L: - Irregular respiration during the third and fourth hour of exposure: 2 males and 2 females.	CA 5.2.3/02 (1987e)

Table 62: Summary table of human data on STOT SE

Type of data/report	**Test substance**	**Route of exposure** **Relevant information about the study**	**Observations**	**Reference**
No human data are available				

Table 63: Summary table of other studies relevant for STOT SE

Type of study/data	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
With the exception of the acute oral, dermal and inhalation toxicity studies summarised in Sections 3.1, 3.2 and 3.2; and the mouse bone marrow micronucleus assay summarised in Section 3.8, no other studies relevant to STOT-SE are available.				

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

In an acute oral (gavage) neurotoxicity study in the rat a decrease in total and ambulatory motor activity counts was observed at 1000 and 2000 mg/kg bw. No effects on neurobehaviour (FOB), neurohistopathology, brain weights or brain dimensions were observed in either sex. Effect level for general toxicity of 2000 mg/kg bw was determined for this study in males and females base on clinical signs (CA 5.7.1/02, 2011a).

No relevant findings indicating serious or severe toxicity were seen in the acute oral, dermal or inhalation toxicity studies, in the mouse micronucleus assay or in the range-finding study for the acute neurotoxicity study (CA 5.7.1/01, 2010).

The acute and repeated exposure inhalation toxicity studies do not indicate any notable respiratory irritation.

10.11.2 Comparison with the CLP criteria

Specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed and not specifically addressed in other sections are included. Assessment takes into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs. Specific target organ toxicity can occur by any route that is relevant for humans, i.e. principally oral, dermal or inhalation. The hazard class is differentiated into three categories.

Substances are classified in Category 1 on the basis of reliable and good quality evidence from human cases or epidemiological studies; 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. Substances are classified in Category 2 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. Category 1 and 2 are assigned on the basis of findings of 'significant' or 'severe' toxicity. In this context 'significant' means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. 'Severe' effects are generally more profound or serious than 'significant' effects and are of a considerably adverse nature with significant impact on health. Both factors have to be evaluated by weight of evidence and expert judgement. For each category, attempts shall be made to determine the primary target organ of toxicity and to classify for that purpose. The data shall be carefully evaluated and, where possible, secondary effects should not be included. The relevant route or routes of exposure by which the classified substance produces damage shall be identified.

Category 3 (transient target organ effects) 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. The criteria for classifying substances as Category 3 for respiratory irritant effects (characterized by localized redness, oedema, pruritis and/or pain) that impair function with symptoms such as cough, pain, choking, and breathing difficulties are included. This evaluation will be based primarily on human data. Subjective human observations could be supported by objective measurements of clear respiratory tract irritation such as electrophysiological responses, biomarkers of inflammation in nasal or bronchoalveolar lavage fluids. The symptoms observed in humans shall also be typical of those that would be produced in the exposed population rather than being an isolated idiosyncratic reaction or response triggered only in individuals with hypersensitive airways. Ambiguous reports of irritations are excluded as this term is commonly used to describe a wide range of sensations which are outside the scope of classification for respiratory tract irritation. Useful information may be obtained from the single and repeated inhalation animal studies. This special classification applies only when more severe organ effects including in the respiratory system are not observed. The criteria for classifying substances as Category 3 for narcotic effects are central nervous system depression including narcotic effects in humans such as drowsiness, narcosis, reduced alertness, loss of reflexes, lack of coordination and vertigo. These effects can also be manifested as severe headache or nausea, and can lead to reduced judgment, dizziness, irritability, fatigue, impaired memory function, deficits in perception and coordination, reaction time, or sleepiness. Narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia. If these effects are not transient in nature, then they shall be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure.

A number of single dose toxicity studies are available for pyriproxyfen: these studies consistently show low acute toxicity. In the acute neurotoxicity study, a decrease in total and ambulatory motor activity counts was observed in male rats only.

Table: Overview of motor counts in male rats (CA 5.7.1/02)

Parameters	♂ (mg/kg bw)				Historical control range[a]
	0	300	1000	2000	
Day 0 – Total locomotor activity counts					
- 0-10 mins	1142	1080	932	945	1064 – 1190 mean: 1134
- 11-20 mins	528	500	349	331	444 – 721 mean: 562
- 21-30 mins	152	146	80	146	102 – 426 mean: 243
- 31-40 mins	88	83	87	90	58 – 188 mean: 128
- 41-50 mins	102	157	98	62	51 – 161 mean: 101
- 51-60 mins	143	166	159	120	51 – 240 mean: 155
- Cumulative	2155	2133	1704	1694*	2109 – 2638 mean: 2323
Day 0 – Ambulatory locomotor activity counts					
- 0-10 mins	341	318	267	248*	290 – 348 mean: 321
- 11-20 mins	88	63	55	44	57 – 152 mean: 95
- 21-30 mins	5	2	13	11	2 – 74 mean: 27
- 31-40 mins	1	2	1	11	0 – 20 mean: 8
- 41-50 mins	4	8	6	0	1 – 13 mean: 5
- 51-60 mins	7	7	14	10	0 – 43 mean: 15
- Cumulative	445	401	356	323*	418 – 582 mean: 471

* $p \leq 0.05$

a laboratory historic control data (Rat CRL:CD(SD) . Range: min.- max. (refer to KCA 5.7.1/02, Appendix K) collected 2007 - 2010

No effects on neurobehaviour (FOB), neurohistopathology, brain weights or brain dimensions were observed in either sex in this study. Considering no effects on neurobehaviour, neurohistopathology or brain weight were observed, and the decrease in motor activity was only observed in males, this finding is not considered severe enough for classification with STOT-SE. In the absence of any indication of significant and/or severe toxic effects of relevance to human health, classification of pyriproxyfen for STOT SE in Category 1 or 2 is not required.

In the absence of any evidence for narcotic effects or evidence of notable respiratory irritation, classification of pyriproxyfen for STOT SE in Category 3 is not required.

10.11.3 Conclusion on classification and labelling for STOT SE

Pyriproxyfen does not require classification for STOT SE according to the CLP Regulation.

10.12 Specific target organ toxicity-repeated exposure

Table 64: 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**
OECD 407 Rat (SD) 12/sex/dose	S-31183 (pyriproxyfen) 97.2% purity Oral (dietary) 0, 300, 1000, 3000,	Changes in serum biochemical parameters indicative of liver injury, increased liver weight and histopathological findings in the liver. Effect level: 1000 ppm (97.6/95.8 mg/kg bw/day)	CA 5.3.1/01 (1998a)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Deviations: no arena observations, sensory reactivity, grip strength or motor activity measured.	10000 ppm (0, 29.3, 97.6, 286 and 913 mg/kg bw/day in males; 0, 28.8, 95.8, 286 and 869 mg/kg bw/day in females) 28 days	NOAEL: 300 ppm (29.3/28.8 mg/kg bw/day) 1000 ppm: - Increased serum cholesterol: +17% males (sign); +11% females (not sign) - Increased albumin: +3% males (sign) and +9% females (not sign); increased A:G ratio: +14% males (sign), +2% females (not sign); decreased triglyceride level males: -25% (sign) 3000 ppm: - Decreased body weight and body weight gain (-5%, sign on day 28) in males. - Increased serum cholesterol: +37% males (sign), +14% females (not sign). - Increased albumin: +3% males (sign) and +9% females (sign) - Increased A:G ratio: +17% males (sign) and +28% females (sign) - Decreased triglyceride males -40% (not sign) and increased triglyceride females (+50%, not sign). - Increased liver weight: around +20% (sign) 10000 ppm: - Clinical signs: loss of hair 6/12 males and 4/12 females; soft stools 12/12 (M and F). - Decreased body weight and body weight gain in males (-7%, sign) and females (-11%, sign). - Decreased food consumption during first week of study in males (-33%) and females (-36%). - Increased water consumption during first three weeks in males (+31%) and during first two weeks in females (+23%). - Increased serum cholesterol: +113% males (sign), +75% females (sign) - Increased albumin: +12% males (sign), +17% females (sign) - Increased A:G ratio: +18% males (sign), +22% females (sign) - Triglyceride decreased in males (-40%, sign) and increased in females (+450%, sign) - Increased relative kidney weight: +10% males (sign) and +12% females (sign) - Increased liver weight: +50% males and females (sign) - Liver enlargement (10M, 9F) and white foci (10M, 3F) - Hepatocellular hypertrophy: 12M, 1F	
OECD 409 Dog (Beagle)	S-31183 (pyriproxyfen) 97.2% purity	Increased liver weight, centrilobular hypertrophy	CA 5.3.1/02 (1987)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
2/sex/dose Deviations: no functional observations.	Oral (capsule) 0, 100, 300, 1000 mg/kg bw/day 28 days	Effect level not determined due to small group size (range finding study). 100 mg/kg bw/day - Increased liver weight: +20%M, +27%F 300 mg/kg bw/day - Increased liver weight: +15%M, +27%F 1000 mg/kg bw/day - Increased liver weight: +28%M, +40%F - Hepatocellular hypertrophy: 2/2 M and 2/2F.	
Comparable to OECD 408 Rat (Crl:CD® BR) 10/sex/dose Deviations: no functional observations, several organ weights not determined, no reticulocyte count.	S-31183 (pyriproxyfen) 95.3% purity Oral (dietary) 0, 400, 2000, 5000, 10000 ppm (23.5, 118, 309 and 642 mg/kg bw/day in males; 27.7, 141, 356 and 784 mg/kg bw/day in females) 90 days	Decreased body weight and body weight gain at 5000 and 10000ppm; changes in serum biochemical parameters indicative of liver injury, increased liver weight and cytoplasmic content of hepatocytes at ≥2000 ppm. Effect level: 2000 ppm (118/141 mg/kg bw/day) NOAEL: 400 ppm (23.5/27.7 mg/kg bw/day) 2000 ppm - Decreased erythrocytes (-8%, sign) hemoglobin (-6%, sign) and hematocrit (-6%, sign) in males - Increased total cholesterol (+48%M) and phospholipids (+32%M) - Increased liver weight: +20%M relative, +15%M absolute. - Increase cytoplasmic content hepatocytes (6M, 6F) 5000 ppm - Reduced body weight gain (-8%) - Decreased erythrocytes (-9%M, -6%F, sign), hemoglobin (-5%M, -6%F, sign) hematocrit (-6%M, -7%F, sign), increased MCH (+3%M, sign) - Increased total cholesterol (+95%M, +40%F) and phospholipids (+76%M) - Increased liver weight: absolute M+31%, F+18%; relative M+45%, F+32% - Increase cytoplasmic content hepatocytes (10M, 7F) 10000 ppm - Reduced body weight gain (-12%) - Decreased erythrocytes (-6%M sign, -5%F not sign), hemoglobin (-3%M not sign, -7%F sign) hematocrit (-5%M, -8%F, sign); decreased MCV in females (-4%, sign); increased MCH males (+4% sign); - Increased BUN (+14%, sign) and creatinine (+17%, sign) females; increased total protein (+9%M, +11%F, sign), albumin (+14%M, +19%F, sign) and calcium (+5%M not sign, +6%F sign)	CA 5.3.2/01 (1989)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		- Increased total cholesterol (+106%M, +77%F) and phospholipids (+78%M, +55%F), increased GGT (+100%M, +300%F) - Bile duct hyperplasia in one female - Increased liver weight: absolute M+43%, F+39%; relative M+66%, F+62%. - Increase cytoplasmic content hepatocytes (9M, 9F) and hepatocellular necrosis (2M, 2F)	
US EPA OPPTS 870.7800 Mouse (Crl:CD1) (ICR) 10 females/dose Immunotoxicity study Deviations: none	Pyriproxyfen 99.5% purity Oral (dietary) 0, 1000, 2000, 5000 ppm (228, 449 and 1139 mg/kg bw/day) 28 days	Reduced weight gain at 5000 ppm. Increased absolute and/or relative liver weights in all treated groups. Effect level for general toxicity: 2000 ppm (449 mg/kg bw/day); NOAEL 1000 ppm (228 mg/kg bw/day) Effect level for immunotoxicity >5000 ppm (1139 mg/kg bw/day) 2000 ppm: - Increased water consumption (+43%, sign) - Increased liver weight: +16% rel, +20% abs 5000 ppm: - Increased water consumption (+43%, sign) - Reduced body weight gain (-31%, sign) - Increased liver weight: +26% rel, +25% abs	CA 5.8.2/01 (2011)
OECD 424 (1997) Rat SD 12/sex/dose Neurotoxicity study Deviations: none	Pyriproxyfen T.G. 99.5% purity Oral (dietary) 0, 1500, 5000, 15000 ppm (equivalent to 0/0, 108/120, 359/407, 1111/1212 mg/kg bw/day 90 days	Lower mean body weights (-16% M, -11%F) and body weight gains (-20%M, -23%F) at 15000 ppm. No treatment-related differences in either neurobehavioral evaluations (FOB, motor activity) or neuropathology were observed in either sex. Effect level (general toxicity): 15000 ppm (1111 / 1212 mg/kg bw/day in males and females, respectively); NOAEL 5000 ppm (359/407 mg/kg bw/day in males and females respectively). Effect level (neurotoxicity): >15000 ppm (1111 / 1212 mg/kg bw/day in males and females, respectively)	CA 5.7.1/03 (2011b)
Comparable to OECD 408 Rat (Crj:CD (SD)) 21/sex/dose Deviations: Haematology and clinical chemistry only performed at sacrifice;	S-31183 (pyriproxyfen) 97.2% purity Oral (dietary) 0, 80, 400, 2000, 10000 ppm (4.8, 24, 121, 682 mg/kg bw/day in males; 5.36, 27.5, 136, 688 mg/kg bw/day in females) 180 days	Changes in serum biochemical parameters indicative of hepatobiliary effects (cholestasis and renal injury). Increased liver weight, and changes in haematological parameters. Effect level: 2000 ppm (121/136 mg/kg bw/day in males and females, respectively); NOAEL 400 ppm (24/27.5 mg/kg bw/day in males and females, respectively). 2000 ppm: - Significantly decreased erythrocytes (-3%M), haemoglobin (-3%M), haematocrit (-3%M, -6%F)	CA 5.3.2/02 (1988b)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
urinalysis only at week 26.		- Increased serum cholesterol (+38%M) and phospholipids (+30%M); reduced serum beta-globulin (-6%M). - Cholestasis and renal injury - Increased relative liver weight M (+9%) 10000 ppm: - Decreased body weight (-13%M, -12%F) - Significantly decreased erythrocytes (-6%M and F), haemoglobin (-3%M, -7%F), haematocrit (-3%M, -6%F) - Increased serum cholesterol (+95%M, +48%F) and phospholipids (+80%M, +20%F); decreased serum beta-globulin (-12%M, -8%F). - Increased liver weight: absolute +34%M, +28%F; relative +56%M and F. - Blackish brown coloration liver (20/21M); hepatocellular hypertrophy in all M and F.	
Comparable to OECD 409 Dog (Beagle) 4/sex/dose Deviations: No functional observations were performed.	S-31183 (pyriproxyfen) 97.2% purity Oral (capsule) 0, 100, 300, 1000 mg/kg bw/day 90 days	Increased liver weight and histopathological changes (hepatocellular hypertrophy). Effect level: 300 mg/kg bw/day in males and females; NOAEL of 100 mg/kg bw/day. 300 mg/kg bw/day: - Increased serum cholesterol (+26%) and phospholipids (+12%). - Increased liver weight: absolute +30%M (sign) and +12%F (not sign); relative +23%M (sign) and +15%F (not sign). - Hepatocellular hypertrophy (3F) 1000 mg/kg bw/day - Clinical signs (soft faeces/diarrhoe in 3F). - Increased platelet count F (+30%, not sign). - Increased alkaline phosphatase (+19%M) and lactate dehydrogenase (+28%M). - Increased serum cholesterol (+23%) and phospholipids (+23%). - Increased liver weight: absolute +26%M (sign) and +11%F (not sign); relative +20%M (not sign) and +16%F (not sign). - Enlarged liver (2M, 1F), hepatocellular hypertrophy (all M and F), eosinophilic bodies (2M, 2F).	CA 5.3.2/03 (1988)
OECD 452 Dog (Beagle) 4/sex/dose Deviations: electrocardiogra	S-31183 (pyriproxyfen) 94.9% purity Oral (capsule) 0, 30, 100, 300, 1000 mg/kg bw/day	Increased liver weight and histopathological changes (cholestasis and hepatocellular injury) seen in males at all dose levels, in females ≥100 mg/kg bw/day.	CA 5.3.2/04 (1991)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
phy was not performed.	52 weeks	Changes in haematological parameters suggestive of anaemia in males ≥300 mg/kg bw/day in females ≥100 mg/kg bw/day. Effect level: 30/100 mg/kg bw/d in males and females, respectively 30 mg/kg bw/day - Increased liver weight: absolute +30%M and +7%F (both not sign); relative +29%M (sign) and +4%F (not sign) - Increased cholesterol: +50%M (not sign). 100 mg/kg bw/day - Decreased erythrocytes and haemoglobin in females (78-90% of control) after 12 and 37 weeks of treatment. Increased MCV in females (+7%). - Increased cholesterol: +157%M - Increased liver weight: absolute +47%M (sign) and +25%F (not sign); relative +58%M (sign) and +36%F (not sign) 300 mg/kg bw/day - Decreased body weight gain M (-49%). - Decreased erythrocytes (-13%) and haemoglobin (-7 to -22%) in males and females. Increased MCV in males and females (+6%). Increased cholesterol in males (+143%) and triglycerides (+88%). - Significantly increased liver weight in males and females: absolute +68%M, +44%F; relative +92%M, +57%F. 1000 mg/kg bw/day - Mortality: 2 males killed in extremis in week 17 and 31. - Salivation, emesis and diarrhoea in M and F - Decreased body weight gain M (-90% day 0-91, recovered afterwards) and F (-50%). - Decreased erythrocytes (-11%) and haemoglobin (-6% to -16%) in males and females. Increased MCV in males and females (+6%, sign). - Significantly increased liver weights: absolute +9%M, +39%F; relative +100%M, +53%F. - Large liver (2M), irregular surface liver (2F and 1 M); bile duct hyperplasia (2/2M, 3/4F), centriacinar fibrosis (2/2M, 3/4F), nodular hyperplasia (2/2M), active chronic	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		inflammation (2/2M, 2/4F), cystic degeneration (1M, 1F); Gall bladder submucosal fibrosis (2/2M and 3/4F).	
OECD 452 Dog (Beagle) 4/sex/dose Deviations: electrocardiography was not performed.	S-31183 (pyriproxyfen) 94.5% purity Oral (capsule) 0, 3, 10 mg/kg bw/day 52 weeks	No significant effects at the highest dose level. Effect level: >10 mg/kg bw/day in males and females	CA 5.3.2/05 (1993)
OECD 453 (1981) Rat Crl:CD® BR (SD) 50/sex/dose Deviations: clinical pathology on satellite group (10/sex/dose) at week 13, 26, 52, 78 and 104. At week 52, 10 animals/sex/satellite group were necropsied.	S-31183 (pyriproxyfen) 95.3% purity 0, 120, 600, 3000 ppm for 104 weeks, equivalent to 0/0, 5.4/7.0, 27.3/35, 138/182.7 mg/kg bw/day	Systemic NOAEL set at 600 ppm (27.2 mg/kg bw/day). 3000 ppm: - Decreased body weight (females: -14% week 50 and -7% week 102; males -5% week 50 and -1% week 102). - Decreased body weight gain females (-9.5% week 1-102). - Increased liver weight: absolute +27% M, +7% F (not statistically significant); relative to bw +13% M, +27% F (not statistically significant). - Increased cholesterol in M (significant in week 26 and 52 (around +46%) ; not significant in week 78 and 104. In F significant increase week 26 only (+46%). - Increased GGT in F in weeks 26 and 52; in M in week 104. - Increased ALP in M +46% week 26 (sign), +49% week 52 (sign), +65% week 78 (sign) and +11% week 104 (not sign). - Increased dark areas liver F (11/34 vs. 1/21 in control) - Increased liver necrosis in unscheduled deaths M 8/23 and F 4/16.	CA 5.5.1/01 (1991a, 1994)
OECD 451 (1981) Mouse Crl:CD-1 (ICR)BR 60/sex/dose Deviations: clinical haematology evaluation performed on	S-31183 (pyriproxyfen) 95.3% purity 0, 120, 600, 3000 ppm (0/0, 16.4/21.1, 81.3/107.3, 422.5/532.8 mg/kg bw/day)	A systemic LOAEL was set at 120 ppm (16.4 mg/kg bw/day) based on the reduced survival rate seen at all dose levels. 120 ppm: - Decreased survival rate: M 45% (control group 57%); , F, 56% (control group 61%). 600 ppm: - Decreased survival rate (M 28%, F 45%) - Increased liver weight: interim absolute +15% F (sign); terminal not significant (absolute +7% M	CA 5.5.1/02 (1991b, 1994)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
10/sex/dose in week 52 and 78.		and F, relative +6% M) - Increased severity of systemic amyloidosis in several tissues (see table B.6.5-12 in Annex I section 3.9.1.2 for details). 3000 ppm: - Decreased survival rate (M 18%, F 36%) - Increased liver weight: F absolute interim significant (+31% absolute, +23% relative); terminal not significant (relative + 5%). - Increased severity of systemic amyloidosis in several tissues (see table B.6.5-12 in Annex I section 3.9.1.2 for details).	
OECD 410 Rat (SD) 5/sex/dose Deviations: none	S-31183 (pyriproxyfen) 97.2% purity Dermal 21 days (6h/day) 0, 100, 300, 1000 mg/kg bw/day	No significant effects at the highest dose level. Effect level: >1000 mg/kg bw/day in males and females	CA 5.3.3/01 (1993)
OECD 412 Rat (SD) 10/sex/dose Deviations: none	S-31183 (pyriproxyfen) 97.0% purity Inhalation 28 days (whole body, 4h/day) 0, 269, 482, 1000 mg/m^3	Males and females: salivation (1-3 animals per sex during the first five days of study), reduced bodyweight gain (-12%M; -7%F). Males: elevated serum LDH activity (+44%, sign) Effect level: 1000 mg/m^3 (~180 mg/kg bw/day)	CA 5.3.3/02 (1988)

Table 65: Summary table of human data on STOT RE

Type of data/report	**Test substance**	**Route of exposure** **Relevant information about the study**	**Observations**	**Reference**
No data are available				

Table 66: Summary table of other studies relevant for STOT RE

Type of study/data	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
No data are available				

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

Four weeks of dietary exposure of rats to 0, 300, 1000, 3000 or 10000 ppm (dietary concentrations equal to mean achieved intakes of 0, 29.3, 97.6, 286 and 913 mg/kg bw/day in males; 0, 28.8, 95.8, 286 and 869 mg/kg bw/day in females) resulted in changes in clinical biochemistry parameters indicative of liver injury at concentrations of ≥1000 ppm. Changes included increased levels of serum cholesterol, calcium and albumin and increased A:G ratio and decreased levels of triglycerides. At higher dietary concentrations, changes in phospholipids, GGT, serum cholinesterase activity, aspartate aminotransferase were noted. Furthermore, at higher dietary concentrations, reduced body weight (3000 and 10000 ppm), anaemia (10000 ppm), an increased incidence of enlargement and white foci of the liver (10000 ppm) and hepatocellular hypertrophy (10000 ppm) were observed. The NOAEL in this study was set at 300 ppm (29.3/28.8 mg/kg bw/day). The effect level identified in this study was 1000 ppm, equal to 97.6 and 95.8 mg/kg bw/day in males and females respectively (CA 5.3.1/01, 1998a).

Four weeks exposure of dogs to 0, 100, 300 or 1000 mg/kg bw/day of pyriproxyfen technical via capsule resulted in increased liver weight in all dose groups and centrilobular hypertrophy at the highest dose level of 1000 mg/kg bw/day (CA 5.3.1/02, 1987). An effect level or NOAEL was not determined for this study due to the small group size (range finding study).

No evidence of immunotoxicity was seen in a study in the female mouse performed at dietary concentrations of 0, 1000, 2000 and 5000 ppm (equivalent to achieved intakes of 0, 228, 449 and 1139 mg/kg bw/day). The effect level for general toxicity following 28 days of continuous dietary is considered to be 2000 ppm (equivalent to 449 mg/kg bw/day) as shown by increased water consumption and increased relative and absolute liver weights. There were no treatment-related effects on the immune function, as assessed by the measurement of antigen-specific, T-cell dependent antibody formation. The effect level for immunotoxicity in this study was therefore greater than 5000 ppm (1139 mg/kg bw/day), a dose which exceeds the maximum recommended dose (1000 mg/kg bw/day) for repeat dose toxicity studies (CA 5.8.2/01, 2011).

Dietary exposure of rats to 0, 400, 2000, 5000 or 10000 ppm pyriproxyfen technical (dietary concentrations equal to mean achieved intakes of 0, 23.5, 118, 309 and 642 mg/kg bw/day for males; 0, 27.7, 141, 356 and 784 mg/kg bw/day for females) for 13 weeks caused reduced body weight gain at ≥5000 ppm. Erythrocyte counts, haemoglobin and haematocrit were decreased in males at ≥2000 ppm and in females at ≥5000 ppm. Mean cell volume was decreased in females at 10000 ppm and mean cell haemoglobin was increased in males at ≥5000 ppm. Blood urea nitrogen and creatinine were increased in females at 10000 ppm. Total protein, albumin and calcium were significantly increased at 10000 ppm, probably caused by dehydration. Total cholesterol and phospholipids were increased at ≥2000 ppm, pointing to disturbed lipid metabolism. Gamma-glutamyltransferase activity was increased at 10000 ppm, indicative of hepatobiliary effects; bile duct hyperplasia was also observed in one female. Liver weight was increased at ≥2000 ppm. An increase in the cytoplasmic content of hepatocytes at ≥2000 ppm, mid-zonal hepatocellular necrosis in two males was also observed. The effect level in this study was established at 2000 ppm, equal to 118 and 141 mg/kg bw/day in males and females respectively (CA 5.3.2/01, 1989).

A dietary 90-day neurotoxicity study confirmed a lack of neurological effects (neurobehavioural and neuropathology), with an effect level for neurotoxicity in this study is considered to be >15000 ppm for both sexes (equivalent to 1111/1212 mg/kg bw/day for males / females), a dose which exceeds the maximum recommended dose (1000 mg/kg bw/day) for repeat dose toxicity studies. Based on the result of this study, the effect level for general toxicity following 90 days of continuous dietary exposure is considered to be 15000 ppm (equivalent to 1111 / 1212 mg/kg bw/day for males / females), as shown by lower mean body weights and body weight gains. It can be concluded that pyriproxyfen is considered not to be a neurotoxicant (CA 5.7.1/03, 2011b).

Dietary exposure of rats to 0, 80, 400, 2000 or 10000 ppm of pyriproxyfen technical (equal to 0, 4.8, 24.0, 121 and 682 mg/kg bw/day for males and 0, 5.36, 27.5, 136 and 688 mg/kg bw/day for females) for 6 months resulted in reduced body weight gain at 10000 ppm. Decreases in erythrocyte count, haemoglobin and haematocrit was observed at ≥2000 ppm in males and at 10000 ppm in females. Mean cell haemoglobin concentration was decreased in females at ≥2000 ppm, while mean cell haemoglobin was increased in males

at 10000 ppm. Platelet count was decreased in females at 10000 ppm. Serum total protein, albumin and calcium were increased at 10000 ppm, consistent with dehydration. Dehydration was also apparent from serum hypernatraemia and increased blood urea nitrogen at 10000 ppm. Decreased β-globulin levels at ≥2000 ppm may reflect perturbations in β-lipoprotein synthesis in the liver. Increased α_2-globulin levels together with proteinuria point to injury of the glomerular membrane. Hypokalaemia in conjunction with higher urinary potassium output is also indicative of kidney effects. Cholesterol and phospholipids were increased at ≥2000 ppm. γ-glutamyl peptidase was increased at 10000 ppm in males pointing to hepatobiliary effects and cholestasis. Reduced cholinesterase activity, aspartate aminotransferase activity and alanine aminotransferase activity confirm disturbed liver function. Concomitantly, liver weights were increased at ≥2000 ppm, macroscopic examination showed the liver to be blackish-brown at 10000 ppm and microscopically hypertrophy was observed in all animals at 10000 ppm, with necrosis in some animals. The effect level for this study is 2000 ppm, equal to 121 and 136 mg/kg bw/day in males and females respectively (CA 5.3.2/02, 1988b).

Dogs were administered 0, 100, 300 or 1000 mg/kg bw/day of pyriproxyfen technical via capsule for 90 days. Alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities were increased during the whole exposure period relative to the pre-test value at 1000 mg/kg bw/day in males. Serum cholesterol and phospholipid concentrations were increased when compared to the pre-test value in all treated males. Liver weights were increased at ≥300 mg/kg bw/day; hepatocellular hypertrophy was observed at ≥300 mg/kg bw/d. The effect level in this study was identified as 300 mg/kg bw/day, as shown by increased liver weights and histopathological changes in the liver (CA 5.3.2/03, 1988).

Exposure of dogs for one year to dose levels of 0, 30, 100, 300 or 1000 mg/kg bw/day of pyriproxyfen technical via capsule resulted in decreased body weight gain at ≥300 mg/kg bw/day. Erythrocyte count, haemoglobin and haematocrit were decreased and mean cell volume was increased at ≥100 mg/kg bw/day, indicative of mild macrocytic anaemia. Platelet count and prothrombin time were increased at ≥300 mg/kg bw/d. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were significantly increased at 1000 mg/kg bw/day, indicative of liver injury. Alkaline phosphatase (ALP) activity was markedly increased at ≥300 mg/kg bw/day and bilirubin was significantly increased at 1000 mg/kg bw/day, indicative of cholestasis. Cholesterol concentrations were increased in all treated males and females at ≥100 mg/kg bw/day. Triglyceride concentrations were markedly increased at ≥300 mg/kg bw/day. Liver weights were increased in all groups of treated males and at ≥100 mg/kg bw/day in females. Histopathological examination revealed significant liver damage in all but one of the animals at 1000 mg/kg bw/day, characterised by centriacinar fibrosis, bile duct hyperplasia and accompanied by submucosal fibrosis of the gall bladder. Nodular hyperplasia accompanied the more severe hepatic lesions. Based on elevated cholesterol levels and increased liver weights, the effect level in this study was identified as 30 mg/kg bw/day in males. The effect level for females in this study was identified as 100 mg/kg bw/day (CA 5.3.2/04, 1991).

In a supplementary study, dogs were exposed to 0, 3 and 10 mg/kg bw/day of pyriproxyfen technical by capsule for 52 weeks. No consistent treatment-related findings were observed in either dose group; therefore the effect level for this study was >10 mg/kg bw/day for males and females (CA 5.3.2/05, 1993).

Subacute dermal exposure of rats to 0, 100, 300 and 1000 mg/kg bw/day pyriproxyfen did not result in any adverse systemic effects or local effects. The effect level for both systemic and local effects in this study was therefore established as being >1000 mg/kg bw/day (CA 5.3.3/01, 1993).

A subacute inhalation study in the rat used achieved concentrations of 0, 269, 482 and 1000 mg/m^3 pyriproxyfen. Salivation, reduced body weight gain, increased LDH were observed at 1000 mg/m^3, which represents the effect level for this study (CA 5.3.3/02, 1989).

In conclusion, in the sub-acute studies preliminary indications of liver injury (hypertrophy) were observed and in the semi-chronic studies marked liver injury was observed, leading to hepatocellular injury, hepatobiliary effects, cholestasis in rat and dog and secondary renal failure in the rat. In addition, in all rodent studies with oral exposure and dog studies, slight changes in haematological parameters were observed.

10.12.2 Comparison with the CLP criteria

Specific target organ toxicity (repeated exposure) is defined as specific, target organ toxicity arising from a repeated exposure to a substance. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included. However, other specific toxic effects that are specifically addressed by other endpoints are not included. These adverse health effects include consistent and identifiable toxic effects in humans, or, in experimental animals, toxicologically significant changes which have affected the function or morphology of a tissue/organ, or have produced serious changes to the biochemistry or haematology of the organism and these changes are relevant for human health. Assessment takes into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs. Specific target organ toxicity can occur by any route that is relevant for humans, i.e. principally oral, dermal or inhalation. Non-lethal toxic effects observed after a single-event exposure are classified as described in Specific target organ toxicity - Single Exposure and are therefore excluded. Substances are classified as specific target organ toxicants following repeated exposure by the use of expert judgement on the basis of the weight of all evidence available, including the use of recommended guidance values which take into account the duration of exposure and the dose/concentration which produced the effect(s), and are placed in one of two categories, depending upon the nature and severity of the effect(s) observed.

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 repeated exposure are classified in Category 1. Classification is on the basis of reliable and good quality evidence from human cases or epidemiological studies; or 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. Guidance dose/concentration values are provided in order to help in classification

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 repeated exposure are classified in Category 2. Classification is 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. Guidance dose/concentration values are provided in order to help in classification. In exceptional cases human evidence can also be used to place a substance in Category 2.

Attempts shall be made to determine the primary target organ of toxicity and classify for that purpose. Secondary effects are not a basis for classification. The decision to classify at all can be influenced by reference to the dose/concentration guidance values at or below which a significant toxic effect has been observed.

Table 68 presents the results of the repeated dose toxicity studies and the equivalent guidance values for classification into category 1 and 2.

Table 67: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	**Effective dose (mg/kg bw/day)**	**Exposure**	**Equivalent guidance value**	**Classification supported by the study**
CA 5.3.1/01 (1998a)	97.6 (M) 95.8 (F)	28 days	30 mg/kg bw/d (Cat 1) 300 mg/kg bw/d (Cat 2)	Yes (Cat 2)
CA 5.3.1/02 (1987)	-	28 days	30 mg/kg bw/d (Cat 1) 300 mg/kg bw/d (Cat 2)	-
CA 5.3.2/01 (1989)	118 (M) 141 (F)	90 days	10 mg/kg bw/d (Cat 1) 100 mg/kg bw/d (Cat 2)	No

Study reference	Effective dose (mg/kg bw/day)	Exposure	Equivalent guidance value	Classification supported by the study
CA 5.8.2/01 (2011) immunotox rat	449	28 days	30 mg/kg bw/d (Cat 1) 300 mg/kg bw/d (Cat 2)	No
CA 5.7.1/03 (2011b) Neurotox rat	1111 (M) 1212 (F)	90 days	10 mg/kg bw/d (Cat 1) 100 mg/kg bw/d (Cat 2)	No
CA 5.3.2/02 (1988b)	121 (M) 136 (F)	6 months	5 mg/kg bw/d (Cat 1) 50 mg/kg bw/d (Cat 2)	No
CA 5.3.2/03 (1988)	300 (M&F)	90 days	10 mg/kg bw/d (Cat 1) 100 mg/kg bw/d (Cat 2)	No
CA 5.3.2/04 (1991)	30 (M) 100 (F)	12 months	2.5 mg/kg bw/d (Cat 1) 25 mg/kg bw/d (Cat 2)	No
CA 5.3.2/05 (1993)	>10 (M&F)	12 months	2.5 mg/kg bw/d (Cat 1) 25 mg/kg bw/d (Cat 2)	No
CA 5.3.3/01 (1993)	>1000	21 days (dermal)	60 mg/kg bw/d (Cat 1) 600 mg/kg bw/d (Cat 2)	No
CA 5.3.3/02 (1988)	1000 mg/m^3 (1 mg/L, ~180 mg/kg bw/d)	28 days (inhalation)	0.06 mg/L (Cat 1) 0.6 mg/L (Cat 2)	No

The 28-day rat study (CA 5.3.1/01, 1998a) reports effects at dietary concentrations as low as 1000 ppm (equal to dose levels of 95.8-97.6 mg/kg bw/day), a level which is relevant for classification. However, findings at the effect level in this study were limited to a marginal increase in serum albumin and globulin concentrations and altered albumin:globulin ratio in males. There were no changes in clinical chemistry parameters consistent with cell damage and no histopathological evidence of liver toxicity at this dose level. Findings were mild in nature, reflect changes in liver function and are not considered to constitute significant toxicity. Studies of 90-day duration do not report relevant effects at dose levels sufficiently low to result in classification for STOT-RE. Also the 6-month study in mice and 1-year study in dog gave effects levels above dose levels relevant for classification (Haber's rule was applied to determine the specific dose levels for classification).

10.12.3 Conclusion on classification and labelling for STOT RE

Pyriproxyfen does not require classification for STOT-RE according to the CLP Regulation.

10.13 Aspiration hazard

Table 68: Summary table of evidence for aspiration hazard

Type of study/data	Test substance,	Relevant information about the study	Observations	Reference
No data are available				

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

No data are available.

10.13.2 Comparison with the CLP criteria

Consideration of classification for aspiration hazard is only relevant for hydrocarbon substances of low viscosity and does not therefore apply to pyriproxyfen.

10.13.3 Conclusion on classification and labelling for aspiration hazard

Pyriproxyfen does not require classification for aspiration hazard according to the CLP Regulation.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Pyriproxyfen is an insecticidal active substance considered under Directive 91/414/EEC (subsequently Regulation 1107/2009) for representative use as a foliar insecticide to control a range of insects in citrus and pome fruits in the field and in tomatoes and ornamentals in both the field and in the greenhouse.

Available environmental fate and ecotoxicology studies have been considered and summarised in the revised Renewal Assessment Report, November 2018 (volume 3, annex B.8: Environmental fate and behaviour and volume 3, annex B.9: Ecotoxicology).

The key information pertinent to determining the environmental hazard classification for pyriproxyfen is presented below. Unless otherwise stated, these studies were conducted in accordance with GLP and the validity criteria of the respective test guideline, if applicable. Full robust summaries of these studies are presented in Annex 1 to this dossier.

11.1 Rapid degradability of organic substances

Table 69: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Aquatic hydrolysis OECD TG 111; GLP; [phenyl^{14}C] (radiopurity: 98%) and [pyridyl^{14}C]-pyriproxyfen (radiopurity: 97%)	Hydrolytically stable at pH 4, 7 and 9 at 50 °C	Acceptable	Katagi T., Takahashi N. (1989) NNM-90-0013 RAR B.8.2.1.1
Aquatic photolysis OECD TG 316; GLP; [Pyridyl-^{14}C]-pyriproxyfen (radiopurity 97%)	DT_{50}: 10.5 hours = 0.8 days (at 30-50 °N summer, OECD)	7.7% volatilisation at 7 days. The half-life was determined using first-order kinetics. acceptable	Ponte M. (2015) NNM-0087 RAR B.8.2.1.2
Ready biodegradation screening EHWD N0.5, PAB No. 615, BIB No. 392[‘74]; OECD TG 301C; GLP; pyriproxyfen (purity 99.4%)	Not readily biodegradable 0.69% biodegradation after 28 days (based on oxygen consumption)	Reporting is limited. Toxicity control not included. Acceptable (with restrictions) **Key study**	Itoh K., Tanoue A., Matsuda T. (1988) NNM-0064 RAR B.8.2.2.1

Method	Results	Remarks	Reference
Surface water degradation simulation OECD TG 309; GLP; [pyridyl 2,6-^{14}C]-pyriproxyfen (radiopurity: ≥96%) and [phenyl-^{14}C]-pyriproxyfen (radiopurity: ≥97%)	<u>Low dose (0.005 mg/L)</u> DT_{50}: 10.6 days at 12 °C DT_{50}: 5.0 days at 20 °C <u>High dose (0.05 mg/L)</u> DT_{50}: 30.8 days at 12 °C DT_{50}: 14.5 days at 20 °C. Mineralisation after 63 days amounted to: 12.4 and 15.6% AR (pyridyl-label) and 18.2 and 32.4% AR (phenyl-label) in high and low dose systems, respectively.	24 Metabolites detected, of which 6 metabolites in quantities above 10% AR. The latter 6 metabolites were identified. Acceptable **Key study**	Adam D. (2015) NNM-0086 RAR B.8.2.2.2
Water/sediment degradation simulation Guideline; SETAC procedures for assessing the environmental fate and ecotoxicity of pesticides, Section 8.2 (1995); GLP; [pyridyl-^{14}C]-pyriproxyfen (radiopurity: 99.6%) and [phenyl-^{14}C] pyriproxyfen (radiopurity: 99.6%)	<u>Total system</u> DT_{50} = 47.1 (pond) and 59.0 (lake) days at 12°C DT_{50} = 22.2 (pond) and 27.8 (lake) days at 20°C Mineralisation after 100 days: Pond system: 53 and 36% (phenyl- and pyridyl-label, resp.); Lake system: 25 and 11% (phenyl- and pyridyl label, resp.) Non-extractable residues after 100 days: Pond system: 39 and 37% (phenyl- and pyridyl-label, resp.); Lake system: 51 and 31% (phenyl- and pyridyl-label, resp.)	Kinetic modelling performed according to FOCUS guidance (2006, 2011). Pseudo-SFO DT50 presented based on slow phase (*k2*) of DFOP and HC biphasic models. Acceptable	Lewis C. J. (2000a) NNM-0076 and Cooke J. (2016b) NNM-0096 RAR B.8.2.2.3
Stability in air Calculated using the Atkinson equation	DT_{50} = 2.457 hours (0.205 days)	Acceptable	Yoshida M., Kodaka R., Fujisawa T. (2013) NNP-0120 RAR B.8.3

11.1.1 Ready biodegradability

Itoh et al (1988) investigated the ready biodegradability of pyriproxyfen in a GLP-compliant closed bottle test according to EHWD No. 5, PAB No. 615, BIB No. 392, a method comparable to OECD TG 301C. Test solutions containing pyriproxyfen (purity 99.4%; 100 mg/L) and activated sludge inoculum (30 mg/L) were kept in bottles in the dark for 28 days at 24.0 to 25.5°C. Test was conducted in triplicate. Single flasks for the inoculum blank control (inoculum, no test substance), “sterile” control (test substance, no inoculum) and the reference substance (aniline, 100 mg/L) were included. The test solution was stirred continuously. Oxygen consumption was continuously monitored, and actual pyriproxyfen concentrations were determined at test end by HPLC-UV.

The oxygen consumption in the inoculum blank control amounted to 8 mg/ 300 mL, corresponding to 26.7 mg O_2/L, which meets the OECD TG 301C validity criterion of <60 mg O_2/L, and is within the recommended range of 20-30 mg O_2/L. The pass level for the reference substance (60% ThOD) was reached within 7 days, with degradation amounting to 67 and 72% after 7 and 28 days, respectively. Pyriproxyfen hardly degraded with biodegradation amounting to 0.32 and 0.69% after 7 and 28 days, respectively.

Relevant details such as the pH, the method of preparation of test solutions, actual measured dissolved oxygen concentrations, handling and composition of the inoculum were not reported. Therefore, the study was considered as not fully documented and regarded as supplemental during the EU revision in 2017.

As the pH was not reported, it cannot be excluded that the low biodegradability of pyriproxyfen was caused by testing at pH values outside the recommended range of 6-8.5. However, the positive control did meet the validity criterion, and thus a pH effect seems unlikely. The study design did not include a toxicity control, which would have allowed to exclude toxicity of pyriproxyfen to the inoculum. In the RAR, however, a GLP-compliant activated sludge, respiration inhibition test according to OECD TG 209 is available for pyriproxyfen that was assessed as acceptable. At the highest tested concentration of 100 mg/L only 2% inhibition was observed, and the EC50 was reported as >100 mg/L. Therefore, it seems unlikely that the low biodegradability of the test substance was due to toxicity to the inoculum.

Considering all above, pyriproxyfen can be concluded as being not readily biodegradable. The results are considered reliable with restrictions, and can be used for classification purposes.

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

Katagi and Takahashi (1989) investigated the hydrolytic behaviour of [^{14}C]-pyriproxyfen in a GLP-compliant hydrolysis as a function of pH study according OECD TG 111. Tests were conducted with [phenyl-^{14}C] and [pyridyl-^{14}C]-pyriproxyfen (radiopurity: 97% and 98%, resp.) at pH 4, 7 and 9 at 50°C for 7 days in a tier 1 test. The recovery of ^{14}C was 96-109%. Pyriproxyfen was degraded with half-lives of greater than 367 days in all buffer solutions. Pyriproxyfen was concluded to be hydrolytically stable at pH 4, 7 and 9. The study was deemed acceptable in the original EU inclusion (2009) and at renewal (2017). The results are considered reliable without restrictions, and can be used for classification purposes.

11.1.4 Other convincing scientific evidence

No data available.

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

No data available.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available.

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

Biodegradation in surface water

Adam (2015) investigated the aerobic mineralisation of [^{14}C]-pyriproxyfen in surface water under defined laboratory conditions in the dark according to OECD TG 309 (2004). The radiolabelled test item, separately labelled in two positions (pyridyl and phenyl positions; radiopurity ≥95.5% and ≥96.7%, respectively) was applied to natural pond water at nominal test item concentrations of 0.05 and 0.005 mg/L. Additionally, the

high concentration experiment conducted with the pyridyl-label was performed under sterile conditions in order to gain information about abiotic degradability of the test item. The test flasks were incubated in the dark for a period of 63 days at 20.9 ± 0.2°C under aerobic conditions. Radiolabelled benzoic acid (with and without solvent control) was used as reference substance to check for sufficient microbial activity of the test water. Duplicate samples treated with the test item(s) were taken from each test system immediately after treatment (time 0) and after 3, 7, 14, 28, 42 and 63 days of incubation. Aliquots of samples treated with benzoic acid were removed from the flasks and analysed immediately after treatment (time 0) and after 7 and 14 days. Samples were analysed by LSC and HPLC.

Oxygen content ranged 8.42-8.67 mg/L, and mean pH ranged 8.50-8.61. Mass balances of individual replicates were in the range 89.9-109.5% AR, with the exception of the day 42 low dose phenyl-label sample (82.7% AR). The test water was considered to be microbially active, as more than 90% of benzoic acid degraded within 7 days in the solvent control samples, and evolved carbon dioxide was simultaneously observed during the 14-day incubation period in each of the test systems. In the sterile control, hardly any degradation was observed with the amount of pyriproxyfen decreasing form 97.4-98.0% AR at the start to 90.7-95.6% AR at study end (day 63). Three metabolites were detected, i.e. 4'-OH-Pyr, DPH-Pyr and PYPA, reaching maximal amounts of 1.5, 2.0 and 3.8% AR in any replicate during the test. In the non-sterile tests, pyriproxyfen levels decreased from 93.0-105.2% AR at the start to ≤6.8% AR at study end (day 63). At the end of incubation (63 days) CO_2 was evolved to levels of 12.4-15.6% AR in pyridyl-labeled systems, and 18.2 and 32.4% AR in phenyl-labeled systems (high and low dose, respectively), whilst formation of CO_2 was insignificant (0.2% AR) in sterilized systems. 24 metabolites were detected, of which 10 metabolites at >10% AR. Six metabolites found at >10% AR were identified: 4'-OH-Pyr (both labels and doses, max 23.9% AR), DPH-Pyr (both labels and doses, max 42.8% AR), PYPAC (pyridyl label, both doses, max 13.1% AR), PYPA (pyridyl label, both doses, max 51.0% AR), POP (phenyl label, both doses, max 13.0% AR) and 4'-OH-POP (phenyl label, both doses, max 16.5% AR). POPA was identified in phenyl-labelled systems (both doses) but never exceeded 4.9% AR. Four metabolites found at >10% AR were not identified: M12 (pyridyl-label, low dose only, max 15.5% AR in any replicate, replicate mean 11.1% AR), M14 (pyridyl-label, low dose only, max 11.7% AR in any replicate, replicate mean 6.3% AR), M20 (phenyl-label, low and high dose, max 15.9% AR in any replicate, replicate mean 12.1 and 12.6% AR for low and high dose, respectively) and M24 (phenyl-label, high dose only, max 16.1% AR, replicate mean 8.0% AR).

Table 70. Metabolites of pyriproxyfen identified in the surface water simulation degradation study

Abbreviation	Chemical name
4'-OH-Pyr	4-(4-hydroxyphenoxy)phenyl (*RS*)-2-(2-pyridyloxy) propyl ether
DPH-Pyr	4-hydroxyphenyl (*RS*)-2-(2-pyridyloxy) propyl ether
POPA	(*RS*)-2-hydroxypropyl 4-phenoxyphenyl ether
PYPA	(*RS*)-2-(2-pyridyloxy) propanol
PYPAC	(*RS*)-2-(2-pyridyloxy) propionic acid
POP	4-phenoxyphenol
4'-OH-POP	4-hydroxyphenoxy phenol

Thus, in surface water pyriproxyfen showed substantial primary degradation under non-sterile testing conditions, while under sterile testing conditions degradation hardly occurred. Under non-sterile conditions, the mineralization ranged 12.4-32.4% AR depending on the labelling position and applied dose. 24 metabolites were formed, of which 10 in quantities exceeding 10% AR. Six of these were identified, while the remaining 18 metabolites were not further investigated. The SFO half-life (DT_{50}) at 20 °C for pyriproxyfen amounted to 14.5 days for the high dose (0.05 mg/L) and 5.0 days for the low dose (0.005 mg/L) test systems. These

degradation half-live values correspond to 30.8 and 10.6 days, respectively, when normalized to 12 °C. Results are considered reliable without restrictions (Ri = 1), and are used for classification purposes.

Biodegradation in water/sediment systems

Lewis (2000a) investigated the degradation of [^{14}C]-pyriproxyfen in two water/sediment systems according to SETAC Procedures for assessing the environmental fate and ecotoxicity of pesticides, Section 8.2 (1995). This guideline served amongst others as a basis for OECD TG 308 from 2002. The data from this study were analysed by Cooke (2016) using the CAKE v3.2 software package according to guidance provided by FOCUS Degradation Kinetics (2006, 2011).

The sediments were sieved through a 2 mm sieve, and the water through a 0.2 mm sieve. Water/sediment systems (2.5 cm sediment layer – 6 cm water) were equilibrated for 54 days at 20±2°C. The test substance was separately radiolabelled in two positions (pyridyl and phenyl; radiopurity of both 99.6%), and was applied to the water layer at a concentration of 73 µg/L (equivalent to a surface application of 225 g a.s./ha distributed in a 30 cm deep water layer). The water/sediment systems were incubated at 20±2°C in the dark under continuous slight agitation (on an orbital shaker). Humidified CO_2-free air was passed through the headspace of the incubation flasks and volatiles and CO_2 were trapped. Sediment biomass was determined (fumigation extraction) at the beginning and end of the study.

Single flasks were analysed at 0, 1, 2, 3, 7, 14, 50 and 100 days after treatment. The water and sediment layer were separated. The water layer was acidified to pH 3-4 (acetic acid) and extracted three times with ethyl acetate, concentrated and analysed by HPLC-RAM (reversed phase) and TLC (normal phase). Radioactivity was determined by LSC. The sediment was extracted three times with MeOH:water and three times with acetone:acetic acid. The combined extracts were concentrated to the aqueous phase and extracted three times with ethyl acetate after acidification to pH 3-4. Extracts were concentrated prior to analysis by HPLC-RAM (reversed phase) and TLC (normal phase). The sediment (only samples with maximum amount of unextractables) was further extracted by reflux and unextractable residues were fractionated into fulvic and humic acids and humin. Unextractables were determined by combustion/LSC. Radioactivity in trapping solutions was determined by LSC. CO_2 in the NaOH trap was confirmed by $BaCl_2$ precipitation. The recoveries of the concentration procedures were 97-99%. During extraction of the water layer, 2-3% AR was lost as a result of dissolved CO_2 losses (experimentally demonstrated). Identification of pyriproxyfen and metabolites was performed by comparison with reference standards.

Mass balances were 90-98% AR. The radioactivity level in the Mill stream pond water decreased from 57/54% AR (phenyl/pyridyl label) on day 0 to 2.1/3.4% AR on day 100. The radioactivity level in the Emperor Lake water decreased from 73/75% AR (phenyl/pyridyl label) on day 0 to 2.7/27% AR on day 100. The extractable amount of radioactivity partitioning into the Mill stream pond sediment increased to 63/59% AR (phenyl/pyridyl label) on day 7/3 and was 5/20% AR on day 100. The amount of extractable radioactivity in the Emperor Lake sediment increased to 47/52% AR (phenyl/pyridyl label) on day 7/7 and was 11/23% AR on day 100. The unextractable fraction in the Mill stream pond sediment increased to 39/37% AR (phenyl/pyridyl label) on day 50/100 and was 37% AR on day 100. The unextractable fraction (NER) in the Emperor Lake sediment increased to 51/31% AR (PP/PYR) on day 100. CO_2 was a major degradation product (53/36% AR in Mill stream pond after 100 days and 25/11% AR in Emperor Lake after 100 days). Volatile organics were insignificant (≤0.2% AR). Of the unextractable sediment radioactivity, 3.2-5.3% AR was released by reflux extraction, 9.9-16.2% AR, 4.4-8.3% AR and 13-23% AR was associated with fulvic acids, humic acids and humin, respectively.

Pyriproxyfen in Mill stream pond water fell from 56/53% AR (phenyl/pyridyl label) on day 0 to <1% AR on day 14. The level of parent pyriproxyfen in Emperor Lake water fell from 72/74% AR (phenyl/pyridyl label) on day 0 to 3.1/1.7% AR on day 14 and <0.1% AR on day 100. The main metabolites in the Mill stream pond system were 4'-OH-Pyr (max. 4.8/15% AR on day 1/7 in water/sediment and PYPAC (max. 6.8/4.3% AR on day 50 in water/sediment). The main metabolites in the Emperor Lake system were 4'-OH-Pyr (max. 3.0/9.8% AR on day 2/14 in water/sediment), DPH-Pyr (max. 12/3.2% AR on day 2/50 in water/sediment) and PYPAC (max. 24/7.6% AR on day100/100 in water/sediment). Minor (≤1.6% AR in any system/compartment)

identified metabolites were POP, POPA, 4'-OH-POP, PYPAC-Me and PYPA. The sum of unidentified components was ≤3.6/3.0% in water/sediment.

In the derivation of DT_{50} values, Single First Order (SFO) kinetics were preferred, but when the SFO fits did not meet the statistical and visual indicators of goodness of fit recommended by FOCUS kinetics, bi-phasic models, e.g. FOMC (First-Order Multi-Compartment), DFOP (Double First-Order in Parallel) and HS (Hockey-Stick) kinetics were investigated. The degradation DT_{50} values at 20°C for the total (water/sediment) system reported in the RAR amounted to 4.8 (DFOP) and 5.7 (HS) days for the Mill stream pond and the Emperor lake systems, respectively. These are. However, the overall DT_{50} values that consider both the faster initial and the subsequent slower portions of the decline curve. These values do not correspond to SFO kinetics. Pseudo-SFO can be derived from biphasic models using the rules of FOCUS Degradation Kinetics where for the DFOP and HC models the DT_{50} is based on the slow phase *k*2. The *k2* were reported to be 0.03116 and 0.02492 for Mill stream pond and Emperor lake, respectively. This results in pseudo-SFO DT_{50} values of 22.2 days for Mill stream pond and 27.8 days for Emperor lake at 20 °C. Normalization to 12 °C yields degradation DT_{50} values of 47.1 and 59.0 days for the total systems, respectively. It should be noted that these values do not account for the NER, even though NER was not characterized in the study. Considering that at test end, NER amounted to 37% AR in Mill stream pond and 51/31% AR (phenyl/pyridyl label) in Emperor Lake, including the NER as parent substance would lead too much higher DT_{50} values. It is also noted that for the surface water and sediment compartments DT_{50} values were reported in the RAR. However, as these DT_{50} correspond to dissipation and not degradation half-lives, they are not considered relevant for the purpose of classification and labelling and are not further discussed.

The study (Lewis, 2000a)) was deemed acceptable following evaluation and peer review at EU level (2009). The kinetics assessment by Cooke (2016) based on the data generated from Lewis (2000a) was deemed acceptable. Results are considered reliable without restrictions, and can be used for classification purposes.

Biodegradation in soil

A conclusion regarding the rapid degradability of pyriproxyfen can be reached based on aquatic degradation data. Consequently, soil degradation data are not used for classification purposes and are not further discussed.

11.1.4.4 Photochemical degradation

Aqueous photolysis

Ponte (2015) investigated the aqueous photolysis of [pyridyl-^{14}C]pyriproxyfen by determining the photolysis quantum yield at 0.020 µg/mL (with <1% acetonitrile co-solvent) in sterilized phosphate buffer at pH 7. Samples were prepared in quartz tubes (15 mm i.d.) for irradiation with a Suntest CPS+ apparatus equipped with a Xenon lamp with filters blocking infrared light and irradiation below 290 nm. The samples were subjected to continuous irradiation for up to 7 days. The average integrated intensities of the light source for the 290-400 nm and 290-800 nm ranges were 47.0 and 379 W/m^2, respectively. Light exposed samples were placed in a temperature controlled deionized water bath maintained at 25±1°C throughout the study period. Dark control samples in Pyrex tubes wrapped with aluminium foil were placed in an incubator maintained at 25±1°C. Volatile gasses were trapped continuously during the exposure period using ethylene glycol to trap organic volatiles, and two 10% aqueous NaOH solutions to trap CO_2. Chemical actinometer samples (PNAP-PYR) were prepared and exposed concurrently with the test solutions for quantum yield determination.

Duplicate light exposed samples were collected at time 0 and 7 additional time points. In order to evaluate the effect of hydrolysis on the degradation of pyriproxyfen, duplicate dark control samples were also collected at each interval. In light exposed samples, recoveries in the NaOH traps represented an average of 7.3% AR by Day 7, while radiocarbon recovered in the ethylene glycol traps represented 0.4% AR at the end of the study. Radiocarbon recovered in the traps for volatiles in dark control samples represented ≤0.5% AR throughout the study period.

Pyriproxyfen degraded rapidly in light exposed samples and represented 7.1% AR following 40 hours of irradiation, declining below detection after 4 days of continuous irradiation. Pyriproxyfen was stable to hydrolysis and represented 90.4% AR in dark control samples following 7 days of incubation in the dark.

The half-life of pyriproxyfen in light exposed samples was determined as 10.5 hours of continuous irradiation based on the percent pyriproxyfen in solution using pseudo-first order kinetics. The half-life of pyriproxyfen in solar day equivalents is presented below. The quantum yield of pyriproxyfen was determined to be 1.42 x 10^{-2}.

The results are considered reliable without restrictions (Ri = 1) and can be used for classification purposes.

Soil photolysis

A conclusion regarding the rapid degradability of pyriproxyfen can be reached based on aquatic degradation data. Consequently, soil photolysis data are not used for classification purposes and are not further discussed.

11.1.4.5 Summary and discussion on degradation

Pyriproxyfen was shown to be hydrolytically stable under environmental conditions. Photodegradation in water was observed under laboratory condition, but this is likely to be limited under realistic environmental conditions. In a modified MITI I test (OECD TG 301C), degradation amounted to <1% after 28 days and pyriproxyfen is considered not readily biodegradable. In a surface water simulation degradation study (OECD TG 309) conducted with radiolabelled pyriproxyfen mineralisation at test end (63 days) ranged 12.4- 32.4% AR depending on the labelling position and applied dose. Primary degradation of pyriproxyfen was observed in the low and high dose systems with the respective DT_{50} values being 5.0 and 14.5 days at the laboratory test temperature of 20 °C, and 10.6 and 30.8 days when normalized to the European standard temperature of 12 °C. One of the DT_{50} values exceeds the limit of 16 days. In the later study 24 metabolites were detected, of which 10 in amounts exceeding 10% AR at some point during the tests in one or more replicates. Six of these metabolites (>10% AR) were identified as 4'-OH-Pyr, DPH-Pyr, PYPAC, PYPA, POP and 4'-OH-POP. It is noted that some of these metabolites might be prone to classification themselves (see section 11.7.2). There are sediment/water and soil degradation data available, but as preferred aquatic fate data are available, the sediment and soil data are not further considered for classification purposes.

Considering all above, pyriproxyfen is considered as **not rapidly degradable** for classification purposes.

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

11.3.1 Volatilisation

The vapour pressure of pyriproxyfen is $<1.33 \times 10^{-5}$ Pa at 23°C. Henry's law constant is $<7.37 \times 10^{-2}$ Pa m^3 mol^{-1} (23°C). These values demonstrate that pyriproxyfen is non-volatile.

A new *in silico* study (Yoshida, Kodaka and Fujisawa 2013, NNP-0120) was submitted for the renewal of Approval of Pyriproxyfen. It is not necessary to meet GLP requirements for a calculation. A summary is provided below, with a robust summary provided in Annex 1 of this dossier.

The decomposition rate constant of pyriproxyfen in the atmosphere is estimated according to the Atkinson's method, using the Atmospheric Oxidation Program (AOPWIN version 1.92, US EPA). The AOPWIN is a computer program calculating the rate constants of the gas-phase reactions between photochemically produced

hydroxyl radical and organic chemicals, and between ozone and olefinic/acetylenic compounds (not relevant for pyriproxyfen).

The reaction rate constant of pyriproxyfen with hydroxyl radicals was calculated to be 52.2359 × 10^{-12} cm^3 $molecule^{-1}$ sec^{-1}. Assuming that a 12-hour daytime hydroxyl radical concentration is 1.5 × 10^6 molecules cm^{-3}, the corresponding half-life was calculated to be 2.457 hours (0.205 days).

The study conclusion is considered reliable with Ri = 1. Endpoints can be used for classification purposes.

11.3.2 Sorption

Based on batch sorption studies in four soils (according to US EPA Pesticide Assessment Guidelines, Subdivision N, 163-1), the geometric mean Koc value is 20030 L/kg (arithmetic mean 1/n of 1.16), indicating that pyriproxyfen has a low mobility in soil. For more details please refer to the RAR.

11.4 Bioaccumulation

Table 71: Summary of relevant information on bioaccumulation

Method	Results *	Remarks	Reference
Partition coefficient *n*-octanol/water Method EC A.8	Log K_{ow} = 4.85 at pH 5 4.86 at pH 7 4.87 at pH 9 log Pow = 5.37	Acceptable Not acceptable. No details provided, only a value is reported	Bates, M. and Liney, P. (2005b) NNP-0103 RAR B.2.7 ECHA dissemination site (2022)
Fish bioaccumulation US EPA Guideline 165-4; GLP; flow-through; bluegill sunfish (*Lepomis macrochirus*); [pyridyl ^{14}C]-pyriproxyfen (radiopurity: 99.1%) and [phenyl-^{14}C]-pyriproxyfen (radiopurity: 100%)	BCF_K whole fish: 1489 and 1653 L/kg (phenyl- and pyridyl-label, resp.) BCF_{SS} whole fish: 1379 and 1495 L/kg (phenyl- and pyridyl-label, resp.) In fish tissue, pyriproxyfen plus 4'-OH-pyriproxyfen and its conjugates together accounted for 37-70% of the radioactive residue on days 21 and 28 of the uptake phase.	Based on total radioactivity. Since substantial metabolism occurred in fish tissue, the reported ^{14}C BCF values overestimate pyriproxyfen bioaccumulation. The BCF values have not been normalized to 5% lipid content, nor have they been corrected for growth. BCF_{SS} values as reported by applicant. BCF_K values have been derived in this classification proposal. Acceptable (with restrictions) **Key study**	Anonymous (1993); CA 8.2.2.3/01a; NNM-31-0027; RAR B.9.2.8.1 Anonymous (1994); CA 8.2.2.3/01b; NNM-41-0031; RAR B.9.2.8.1 Anonymous (1999); CA 8.2.2.3/01c; NNM-0061; RAR B.9.2.8.1
Fish bioaccumulation EHWD No. 5, PAB 615, BIB 392; GLP; flow-through; carp (*Cyprinus carpio*); pyriproxyfen (purity 99.4%)	BCF_{SSL} whole fish: 669 and 512 L/kg (5 and 0.5 µg/L treatments, resp.)	Based on parent substance. Normalised to 5% lipid content. Several deviations from OECD TG 305, incl. reduced sampling and shorter depuration phase. Lipid normalized BCF_K could not be calculated. Acceptable (with restrictions)	Anonymous (1993a); CA 8.2.2.3/02a; NNR-90-0020; RAR B.9.2.8.2 Anonymous (1993b); CA 8.2.2.3/02b; NNR-0020-1; RAR B.9.2.8.2

Method	Results *	Remarks	Reference
		Key study	

* BCF_K = kinetic BCF; BCF_{SS} = steady-state BCF; BCF_{SSL} = lipid normalised steady-state BCF

11.4.1 Estimated bioaccumulation

As experimental data are available, estimations of bioaccumulation potential not provided.

11.4.2 Measured partition coefficient and bioaccumulation test data

Measured partition coefficient

The log K_{OW} of pyriproxyfen has been experimentally determined to be 4.85 at pH 5, 4.86 at pH 7, and 4.87 at pH 9. Study was not detailed in the RAR. As the experimental log K_{ow} exceeds the threshold of log K_{ow} ≥4, **pyriproxyfen is considered to have a potential for bioaccumulation**.

Bioaccumulation in fish

There are two studies that investigated the bioaccumulation potential of pyriproxyfen in fish. Three additional studies are reported in the RAR that further investigated the data obtained from the two original fish bioaccumulation studies.

Bioaccumulation in bluegill sunfish

Bioaccumulation of ^{14}C-labelled pyriproxyfen was studied in bluegill sunfish (*Lepomis macrochirus*) under flow-through conditions according to US EPA 165-4 (Anonymous, 1993; CA 8.2.2.3/01a; NNM-31-0027). The characterization of ^{14}C-residues in bluegill sunfish was performed under a separate study CA 8.2.2.3/01b (1994, NNM-41-0031), and the clearance time values were calculated in CA 8.2.2.3/01c (1999, NNM-0061). The test substance was separately radiolabelled in two positions (pyridyl and phenyl; radiopurity of 99.1% and 100.0% respectively). Bluegill sunfish (mean weight 0.84 g; length 32.1 mm) were exposed to 0.02 mg/L pyriproxyfen for 28 days to measure uptake of the compound and then placed in clean water for 14 days to determine elimination rate. Solvent control (dimethyl formamide) and treatments (one for each labelling position) consisted of a single 60-L test chamber containing 250 fish. Well water (pH 6.4-7.1; hardness 56-80 mg $CaCO_3$/L) was used as medium. Flow rate was 480 L/ day/ test chamber, resulting in a renewal rate of ~11 volumes/day. Six randomly selected fish were sampled during uptake from the treatment chambers on days 3, 7, 14, 21 and 28, and from the solvent control chamber on days 0, 21 and 28, respectively. During depuration six fish were sampled on days 1, 3, 7, 10 and 14. Water samples were taken at days 0, 3, 7, 14, 21 and 28 during uptake, and on days 1 and 3 of depuration. Of the six fish three were used for whole fish analysis, and three for filler/edible and viscera/non-edible. Additional 85 fish were taken on days 21 and 28 of uptake for determination of metabolites. Radioactivity was analysed by LSC. Fish used for metabolite identification were homogenized and extracted with methanol. Extracts were analysed with TLC and co-chromatography with reference standards. Post extraction solids were analysed by LSC.

During testing temperature ranged 20.4-22.1 ºC, pH 6.4-7.1, and dissolved oxygen concentration 4.7-8.9 mg/L (>68% of saturation). Mortality during the uptake phase was 7% in the control (17 fish) and 4% in each of the treatments (9 and 10 fish). No fish died in the depuration phase. Fish showed no abnormal behaviour. The

mean concentration of pyriproxyfen based on measured radioactivity concentration in the treatment chambers during the exposure phase, was 0.0191 and 0.0200 mg/L for the ^{14}C-phenyl and ^{14}C-pyridyl labelled pyriproxyfen, respectively. Steady-state condition was reached within approximately 7 days. The reported steady-state BCF values based on total radioactivity were for phenyl-^{14}C 1379, 465, and 2482 L/kg for whole body, edible, and non-edible, respectively, and for pyridyl-^{14}C 1495, 478 and 2390, respectively. Pyriproxyfen was reported to be readily cleared away from fish tissues (1.9-10% of ^{14}C residues remaining in whole fish after the 14-day depuration phase) with the CT_{95} calculated to be in the range 4.4-11.0 days. In fish tissue, pyriproxyfen plus 4'-OH-Pyr and its conjugates together accounted for 37-70% of the radioactive residue. Other metabolites were found as conjugates (4'-OH-POP and 5-OH-PYPAC), or in relatively small amounts as simple nonconjugated fragments of pyriproxyfen (POPA, DPH-Pyr, PYPAC, 2-OH-PYPA and PYPA) (see Table 70 for Chemical names). The study noted that the major metabolic process in the fish is hydroxylation followed by conjugation of the hydroxylated products. Both the hydroxylated and conjugated products were eliminated by the fish and were found in the exposure water.

In the RAR it was noted that the lipid content was not determined and that the reported BCF values have not been normalization to 5% lipid. This was considered a major deficiency of the study. Another identified deficiency was that bioconcentration was only determined at one test concentration instead of two, not allowing to conclude if there is a dependency between BCF and exposure concentration . It was further noted that there was no correction for growth rate, which was considered acceptable as body mass apparently did not change substantially over the course of the test (weight at test end was not reported in the RAR). The RAR reports uptake and elimination rates of 1135.8 and 0.8002, respectively, for a BCF of 1419.3. No other details are provided.

It is agreed with the RAR that the presented BCF values are an overestimate as parent (pyriproxyfen) levels in the edible and non-edible fish tissues were determined to be 50% or less on days 21 and 28. However, the reported steady-state BCF values on itself are considered an underestimate. These values were calculated using an average whole fish ^{14}C concentration derived from all uptake phase sampling times (days 3, 7, 14. 21 and 28), while on day 28 in both treatments the highest concentration was reported (respectively 51 and 27% higher than the concentration reported on day 21). This exceeds the OECD TG 305 limit of 20%. For this classification proposal, all the uptake and depuration phase data (as reported in tables B.9.2.8.1-01 and B.9.2.8.1-02 of RAR volume 3 CA B.9) were used to calculate the kinetic BCF values for both treatments (see figure below), yielding whole fish BCF values of 1489 and 1653 L/kg for the phenyl-^{14}C and pyridyl-^{14}C labelled pyriproxyfen treatments, respectively. Assuming that 50% of the radioactivity can be attributed to the parent would still result in BCF values exceeding the trigger of ≥500 L/kg (i.e. whole fish BCF values of 745 and 827 L/kg). The RAR reports parent concentrations for edible and non-edible tissue on days 21 and 28. Unfortunately, the ratio between edible and non-edible tissue is not reported, and therefore no reliable whole fish BCF values for the parent can be calculated. Overall, the data are considered reliable with restrictions (Ri=2) and can be used for classification purposes. **The experimentally determined BCF values of this study indicate a potential for bioaccumulation**.

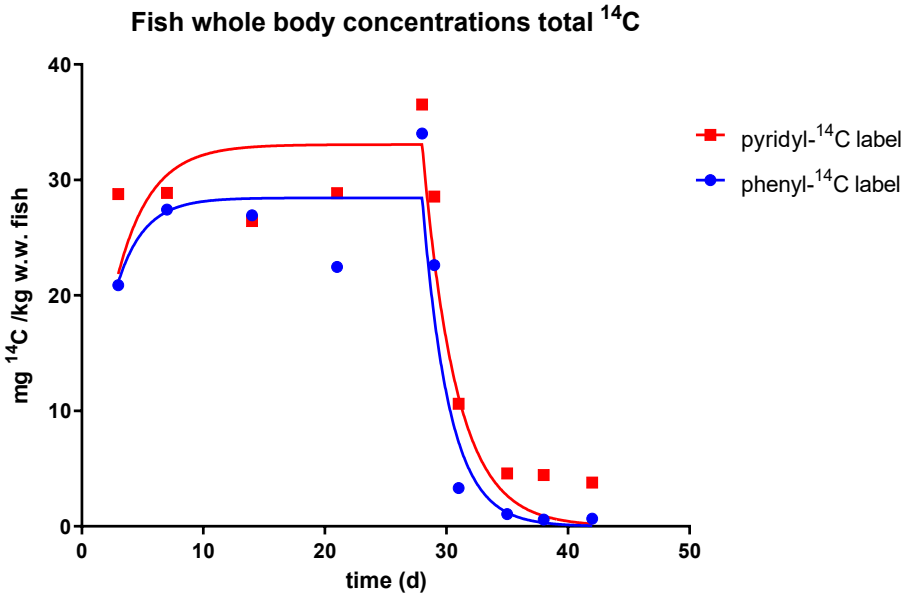

Figure 1. Fish whole body concentrations based on total ^{14}C, including a kinetic BCF fit (based on Anonymous, 1993 (CA 8.2.2.3/01a; NNM-31-0027), as calculated by the Dossier Submitter

Bioaccumulation in carp

Bioaccumulation of pyriproxyfen (purity 99.4%) was studied in carp (*Cyprinus caprio*) under flow-through conditions according to EHWD No.5, PAB No.615, BIB No.392, "Method for Testing the Degree of Accumulation of Chemical Substances in Fish Body", as described in OECD TG 305C (Anonymous, 1993a; CA 8.2.2.3/02a). The excretion study was reported in Anonymous (1993b; CA 8.2.2.3/02b).

Carps (mean weight 22.9 g; length ~10 cm) were exposed to 0.5 and 5.0 µg/L pyriproxyfen for 8 weeks to measure uptake, followed by 7 days to determine depuration. Stock solution was made by dissolving pyriproxyfen in acetone, mixing it with hydrogenated castor oil, evaporating acetone and emulsifying the residue with medium. Solvent control and treatments consisted of a single 100-L aquarium containing 20 fish. Medium was dechlorinated tap water (pH 6.9-7.5). Renewal rate of 14.4 times/day. Fish were sampled every two weeks during uptake phase and on days 1, 2, 4 and 7 of the depuration phase. Four fish were taken at each sampling time, of which two fish were individually analysed for pyriproxyfen and lipid content. Water samples were taken throughout the study twice a week to determine water quality and pyriproxyfen concentrations. Analysis was conducted using liquid chromatography.

The dissolved oxygen concentration ranged from 6.3-8.5 mg/L, pH 6.9- 7.5. The mean measured pyriproxyfen concentrations in water ranged 80-86% and 72-78% for the 0.5 and 5.0 µg/L treatments, respectively. The lipid content of the test fish ranged from 3.0 to 6.3% and linear relationships were established between the lipid contents and the bioconcentration factors at both exposure levels. Fish body weight and length did not increase during the exposure phase. The applicants reported BCF values of 290-850 and 230-900 for the low and high treatment, respectively.

These studies were considered as not acceptable in the RAR, with the main limitations being: the steady state and kinetic BCF values were not calculated in the study report; only two fish per concentration were analysed per each time point, instead of at least four according to the OECD TG 305; the depuration phase lasted 7 days, instead of the half of the duration of the uptake (which totalled 8 weeks); the 7-day depuration phase was considered sufficient to calculate a reliable depuration rate for the low concentration but not for the high concentration. While the RMS calculated stead-state whole fish BCF values normalized to 5% lipid of 512 and 669 L/kg for the low and high treatments, respectively, the BCF values were not used for risk assessment due to the above noted limitations and because reliable higher BCF values were available for bluegill sunfish.

For classification and labelling purposes this study can be used as supplemental data. It is agreed that this older study does not fully comply with current OECD TG 305 requirements, but there are no severe methodological deficiencies that invalidate the study. The issues noted above in the RAR, as well as that there were only four sampling points during uptake phase instead of five, do add to the uncertainties associated with the data. It is also noted that there were only 20 fish per aquarium while 4 fish per sampling would mean there should be 32 fish (in total 8 sampling points). The summary notes that lipid content was determined during uptake and depuration phases. In the RAR lipid corrected pyriproxyfen concentrations in fish are reported for the uptake phase, but neither the lipid content during depuration nor the pyriproxyfen concentrations in fish for the depuration phase have been reported. As the original report was not available (and could not be retrieved from Japan CHEmicals Collaborative Knowledge database (J-CHECK)), these data gaps could not be further investigated. As it would require to estimate the lipid content of individual fish, kinetic BCF values were not calculated for this study (as this would even more increase the uncertainties). **Overall, this study does support that pyriproxyfen has a potential for bioaccumulation**.

11.5 Acute aquatic hazard

The results presented in the table below are from the short-term aquatic toxicity studies conducted with pyriproxyfen. The RAR also reports studies conducted with the formulation Pyriproxyfen 10EC (S-71639). According to the Safety Data Sheet included in the RAR (Volume 3 CP B4) the formulation also contains Hydrocarbons, C10, aromatics, <1% naphthalene (CAS not available) at an amount of ≥10% w/v; 2-ethylhexan-1-ol (CAS 104-76-7) at >1% w/v and calcium dodecylbenzenesulphonate (CAS 26264-06-2) at >1% w/v. As these substances can affect the outcome of the aquatic toxicity tests (i.e. the latter two substances have been self-classified as affecting the aquatic environment), no reliable effect concentrations can be derived for pyriproxyfen from the test performed with the formulation. Therefore, the aquatic toxicity tests conducted with formulation are not further discussed. Summaries of the active substance data are provided in the subsequent sections. For further details, please see Annex 1 to this CLH proposal (section 4.3.1). We note that there are short-term toxicity studies available for degradation products. These are not discussed in this proposal. However, critical long-term aquatic toxicity studies conducted with degradation products are discussed in section 11.6 on chronic aquatic toxicity.

Table 72: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
Fish					
Acute toxicity to fish flow-through US EPA 72-1; GLP	Rainbow trout (*Oncorhynchus mykiss*), old name (*Salmo gairdneri*)	Technical pyriproxyfen Purity: 95.3%	96h-LC_{50}: >0.325 mg/L (mean measured)	Acceptable	Anonymous (1989a) CA 8.2.1/01; NNW-91-0035; RAR B.9.2.1.1
Acute toxicity to fish flow-through US EPA 72-1; GLP	Bluegill sunfish (*Lepomis macrochirus*)	Technical pyriproxyfen Purity: 95.3%	96h-LC_{50}: >0.270 mg/L(mean measured)	Acceptable Key study	Anonymous (1989b) CA 8.2.1/02; NNW-91-0034; RAR B.9.2.1.2
Acute toxicity to fish flow-through	Sheepshead minnow (*Cyprinodon variegatus*)	Technical pyriproxyfen Purity: 95.3%	96h-LC_{50}: >0.328 mg/L (expressed as exceeding	Test concentrations greatly exceed maximal solubility in	Anonymous (1991)

US EPA 72-3; GLP			maximal solubility in the test medium)	filtered natural seawater that was used as medium Acceptable (with restrictions)	CA 8.2.1/05; NNW-11-0070; RAR B.9.2.1.5
Aquatic invertebrates					
Acute toxicity to aquatic invertebrates flow-through US EPA 72-2; GLP	Water flea (*Daphnia magna*)	Technical pyriproxyfen Purity: 95.3%	48h-EC_{50}: 0.40 mg/L (mean measured)	Acceptable	Burgess (1989) CA 8.2.4.1/01; NNW-91-0036; RAR B.9.2.4.1
Acute toxicity to aquatic invertebrates flow-through US EPA 72-3; OPPTS 850.1025; GLP	Eastern oyster (*Crassostrea virginica*)	Technical pyriproxyfen, Purity: 97.1%	96h-EC_{50}: 0.092 mg/L (mean measured)	Acceptable	Dionne (1998) CA 8.2.4.2/01; NNW-0138; RAR B.9.2.4.6
Acute toxicity to aquatic invertebrates flow-through OPPTS 850.1035; GLP	Mysids (*Mysidopsis bahia*)	Technical pyriproxyfen, Purity: 97.1%	**96h-EC_{50}: 0.065 mg/L** (mean measured)	Acceptable **Key study**	Sousa (1999) CA 8.2.4.2/02; NNW-0139; RAR B.9.2.4.7
Algae or other aquatic plants					
Algal growth inhibition static OECD 201; GLP	Green algae (*Raphidocelis subcapitata*)	Technical pyriproxyfen, Purity: 97.2%	72h-E_rC_{50}: 0.111 mg/L 72h-E_bC_{50}: 0.064 mg/L (mean measured)	Acceptable Key study	Blasberg, Hicks and Cramer (1991) CA 8.2.6.1/01; NNW-11-0068; RAR B.9.2.6.1
Lemna growth inhibition semi-static US EPA 122-2 and 123-2; GLP	Duckweed (*Lemna gibba*)	Technical pyriproxyfen, Purity: 98.4%	14d-$EC_{50,\ frond\ density:}$ >0.18 mg/L 14d-$EC_{50,\ biomass}$: >0.18 mg/L (mean measured)	Acceptable	Hoberg (1996) CA 8.2.7/01; NNW-0126; RAR B.9.2.7.1

11.5.1 Acute (short-term) toxicity to fish

There are three studies available in the RAR that investigated the acute toxicity of pyriproxyfen to fish.

Anonymous (1989a) performed a GLP-compliant 96-hours flow-through test with pyriproxyfen (purity of 95.3%) using rainbow trout (*Oncorhynchus mykiss*; old name: *Salmo gairdneri*) according to US EPA 72-1 (CA 8.2.1/01; RAR B.9.2.1.1). Five concentrations were tested with the nominal test concentrations being 22.5, 45, 90, 180 and 360 µg/L. Control and solvent control (solvent not specified) were included. All treatments, including controls, were tested without replicates. Test vessels were 30-L glass aquaria containing 20 fish (initial weight and length not reported). Medium was well water (not further specified). Flow rate was 7.2 volumes/day. Temperature was maintained at 13 °C. Observations and analytical monitoring (GC/LC) were performed after 0 and 96 hours. Dissolved oxygen levels ranged 8.2-8.9 mg/L corresponding to 81 and 88% saturation at 13 °C. pH was not reported. The actual pyriproxyfen concentrations averaged 74 ± 11% and 74 ± 26% of nominal test concentrations at test start and end, respectively. The mean measured test concentrations were: 20.3, 32, 54, 102 and 325 µg/L. The average fish body weight and length of the control treatment were determine at test end amounting to 2.3 ± 1.5 g and 54 ± 3.5 mm, respectively. No mortalities occurred, except for one fish that died in the highest treatment at 96h. Sublethal effects (erratic swimming, loss of equilibrium and surfacing) were observed at the highest treatment. The 96h-LC_{50} of pyriproxyfen was expressed as >325 µg/L based on mean measured test concentrations. Study was considered valid and acceptable in the RAR. The dossier submitter notes that reporting was generally limited lacking relevant data (e.g. water quality details (pH and hardness) not reported, and fish weight and length at test start not reported). Also there were no replicates, but as sufficient number of fish were tested per treatment (>7 as per OECD TG 203), the results can be used for classification purposes.

Anonymous (1989b) performed a GLP-compliant 96-hours flow-through test with pyriproxyfen (purity of 95.3%) using bluegill sunfish (*Lepomis macrochirus*) according to US EPA 72-1 (CA 8.2.1/02; RAR B.9.2.1.2). Five concentrations were tested with the nominal test concentrations being 0.0225, 0.045, 0.090, 0.180 and 0.360 mg/L. Control and solvent control (acetone) were included. All treatments, including controls, were tested without replicates. Test vessels were 40-L glass aquaria filled with 30 L medium and containing 20 fish (mean weight: 1.2 ± 0.77 g; mean length: 34 ± 5.9 mm). Medium was well water (pH 7.1; hardness 40-48 mg $CaCO_3$/L). Flow rate was 7.0 volumes/day. Temperature was maintained at 23 °C. Observations were performed every 24 hours, water quality parameters every 48 hours, and analytical monitoring (method not specified) at test start and end. pH remained 7.1, dissolved oxygen levels ranged 7.8- 8.4 mg/L corresponding to 95 and 102% saturation at 23 °C. The actual pyriproxyfen concentrations averaged 81.4 ± 8.2% and 71.6 ± 4.5% of nominal test concentrations at test start and end, respectively. The mean measured test concentrations were: 0.0191, 0.0315, 0.070, 0.135 and 0.270 mg/L. No mortality or behavioural/sublethal effects were observed in any of the treatments. The 96h-LC_{50} of pyriproxyfen was expressed as >0.270 mg/L based on mean measured test concentrations. Study was considered valid and acceptable in the RAR. The results can be used for classification purposes.

Anonymous (1991) performed a GLP-compliant 96-hours flow-through test with pyriproxyfen (purity of 95.3%) using sheepshead minnow (*Cyprinodon variegatus*) according to US EPA 72-1 (CA 8.2.1/05; RAR B.9.2.1.5). A primary stock solution was prepared in acetone (50 g/L) and further diluted to a secondary stock solution with medium (nominal 5 mg/L; mean measured 1.75 mg/L). White precipitation was observed in the secondary stock solution. Five concentrations were tested with the nominal test concentrations being 0.7, 1.2, 2.0, 3.0 and 5.0 mg/L. Control and solvent control (acetone) were included. All treatments, including controls, were tested in duplicate. Test vessels were 19.6-L glass aquaria filled with 15 L medium and containing 10 fish each (initial weight and length not reported). Medium was filtered natural seawater. Flow rate was 70 volumes/day. Aeration was not applied. Fish were not fed. Temperature ranged 21.0-22.1 °C. Observations and water quality measurements were performed every 24 hours, and analytical monitoring (GC/LC) at test start and end. Salinity was 15-16 ppt; pH ranged 7.8-8.2, dissolved oxygen levels ranged 6.0- 7.7 mg/L temperature ranged 22.2-22.9 °C. At the two highest test concentrations, insoluble test material (surface slick) was immediately observed. After 24 hours, all treatments (except controls) had white precipitation. The actual pyriproxyfen concentrations after 24 hours amounted to 0.164, 0.295, 0.547, 0.906 and 1.47 mg/L, respectively, and after 96 hours to 0.328, 0.303, 0.308, 0.327 and 0.575, respectively. The mean measured test

concentrations were reported as 0.246, 0.299, 0.428, 0.617 and 1.02 mg/L. No mortality or behavioural/sublethal effects were observed in any of the treatments, except for 3 dead fish at 1.02 mg/L treatment (15%) and 1 dead fish in the 0.617 mg/L treatment (5%). The 96h-LC_{50} of pyriproxyfen was expressed as >1.02 mg/L based on mean measured test concentrations. Study was considered valid and acceptable in the RAR. The dossier submitter notes that all test concentrations (0.7 to 5.0 mg/L) greatly exceeded the water solubility of pyriproxyfen that was determined in pure water to be 0.101 mg/L at pH 7. This is reflected by the observations that in the two highest test concentrations a film immediately appeared, while after 24 hours precipitation occurred in all test concentrations. The actual concentration reported after 24 hours in the two highest tests concentrations is clearly an overestimate of the truly dissolved amount of pyriproxyfen, possibly as suspensions were analysed or film was included. The actual concentrations after 96 hours are, except for the highest test concentration, in the range 0.303 to 0.328 mg/L. This appears to be the solubility of pyriproxyfen in the filtered natural seawater that was used as medium (water solubility of pyriproxyfen in pure water is 0.101 mg/L at pH 7). Expressing the LC_{50} as exceeding the solubility in the specific medium seems more appropriate. Considering the too high dosing and potential analytical measurements of suspensions, the results are considered reliable with restrictions provided that the 96h-LC_{50} is expressed as >0.328 mg/L (Klimisch score of 2). The results can be used for classification purposes.

Concluding

From the above summaries it can be concluded that there are three reliable 96h-LC_{50} values available for three different fish species, ranging >0.270 to >0.328 mg/L. Since all three values are greater than values, no conclusions can be drawn with respect to the most sensitive fish species, and consequently which study should be considered as key study. In this specific case, that is not considered an issue, as aquatic invertebrates appear to be a more sensitive taxon than fish.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

There are three studies available in the RAR that investigated the acute toxicity of pyriproxyfen to aquatic invertebrates.

Burgess (1989) performed a GLP-compliant 48-hours flow-through test with pyriproxyfen (purity of 95.3%) using the water flea *Daphnia magna* according to US EPA 72-2 (CA 8.2.4.1/01; RAR B.9.2.4.1). Five concentrations were tested with the nominal test concentrations being 0.06, 0.12, 0.25, 0.50 and 1.0 mg/L. Control and solvent control (acetone) were included. Each treatment consisted of 4 replicates. Test vessels were 1-L glass beakers containing 10 daphnids (age <24 h). Temperature was 20±2 °C. Medium was water with total hardness of 160-180 mg $CaCO_3$/L. Flow-rate was 5.8 volumes/day. Immobility and abnormal effects assessed after 24 and 48 hours. Measurement of water quality parameters and analytical monitoring were performed at 0 and 48 hours. Temperature ranged 20-21 °C, pH ranged 7.9-8.0 and dissolved oxygen from 7.6 to 8.0 mg/L (87 and 94% saturation, respectively). Test water was clear and free of precipitates. The actual pyriproxyfen concentrations averaged 80 ± 8.9% and 66 ± 16% of nominal test concentrations at test start and end, respectively. The mean measured test concentrations were: 0.043, 0.089, 0.19, 0.43 and 0.60 mg/L. No mortality or behavioural/sublethal effects were observed in the controls and lowest treatment of 0.043 mg/L. At 0.089 mg/L 78% of the daphnids showed abnormal behaviour (quiescent/ lying on the bottom), but none of the daphnids were immobile; at 0.19, 0.43 and 0.60 mg/L immobility amounted to 3, 35 and 93%, respectively, while remaining daphnids showed abnormal behaviour. The 48h-EC_{50} of pyriproxyfen was expressed as 0.40 mg/L and the 48h-NOEC as 0.043 mg/L based on mean measured test concentrations. In the RAR a 48h-EC_{50} of 0.46 mg/L was calculated by refitting the data, but it was concluded to use the lowest value of as 0.40 mg/L. Study was considered valid and acceptable in the RAR. The results can be used for classification purposes.

Dionne (1998) performed a GLP-compliant 96-hours flow-through test with pyriproxyfen (purity of 97.1%) using the eastern oysters (*Crassostrea virginica*) according to US EPA 72-3/ OPPTS 850.1025 (CA 8.2.4.2/01; RAR B.9.2.4.6). Prior to testing oysters were acclimatized for 10 days to testing conditions (see below). During testing and acclimation oysters were fed with algae (*Isochrysis galbana*). Prior to testing, 3 mm of the new peripheral shell growth of each oyster was removed, and oysters were observed for signs of stress. Five concentrations were tested with the nominal test concentrations 18, 30, 50, 84 and 140 µg/L. Control and solvent control (acetone) were included. Each treatment was conducted in duplicate. Test vessels were glass

aquaria containing 18 L medium and 20 oysters (reproductively immature; mean valve height of 34 ± 2.8 mm). Medium was natural unfiltered seawater. Flow-rate was ~6 volumes/day. Biological observations (excessive mucous production; faeces production) and water quality monitoring were conducted at start and every 24 hours. Analytical monitoring (GC) and shell deposition (sublethal effect) were determined at test start and end. Temperature ranged 21-22 °C, salinity ranged 31-32‰, pH ranged 7.3-7.7 and the dissolved oxygen concentration ranged 58-85% of saturation (but was generally >60%). The actual pyriproxyfen concentrations averaged 63-89% and 50-79% of nominal test concentrations at test start and end, respectively. The mean measured test concentrations were 13, 17, 38, 53 and 110 µg/L. There were no mortalities. Mean shell growth amounted to 3.3, 3.1, 3.1, 2.9, 3.1, 2.4 and 1.2 mm in the control, solvent control, 13, 17, 38, 53 and 110 µg/L treatments, respectively. The control and solvent control did not differ significantly. The shell growth in the two highest treatments was significantly reduced compared to the pooled control (3.2 mm). Only in the highest treatment was a reduced feeding and faecal matter production observed. The following effect concentrations were reported: 96h-EC_{50} of 92 µg/L; 96h-LC_{50} >110 µg/L and 96h-NOEC of 38 µg/L. Study was considered valid and acceptable in the RAR. The results can be used for classification purposes.

Sousa (1999) performed a GLP-compliant 96-hours flow-through test with pyriproxyfen (purity of 97.1%) using mysid shrimps (*Mysidopsis bahia*) according to OPPTS 850.1035 (CA 8.2.4.2/02; RAR B.9.2.4.7). Five concentrations were tested with the nominal test concentrations 26, 43, 72, 120 and 200 µg/L. Control and solvent control (acetone) were included. Each treatment was conducted in duplicate. Test vessels were glass aquaria containing ~8-11 L medium and 10 mysids (≤24 hours). Medium was natural filtered seawater. Flow-rate was ~6.5 volumes/day. Biological observations and water quality monitoring were conducted at start and every 24 hours. Analytical monitoring (GC) was performed at test start and end. Temperature ranged 24-25 °C, salinity ranged 19-20‰, pH ranged 7.7-7.9 and the dissolved oxygen concentration ranged 77-100% of saturation. The actual pyriproxyfen concentrations averaged 62-75% and 70-105% of nominal test concentrations at test start and end, respectively. The mean measured test concentrations were 19, 38, 61, 80 and 150 µg/L. Mortality amounted to 5, 0, 0, 0, 30, 100 and 100% in the control, solvent control, 19, 38, 61, 80 and 150 µg/L treatments, respectively. Sublethal effects (lethargy) were only observed in the surviving daphnids at 61 µg/L. The following effect concentrations were reported: 96h-LC_{50} of 65 µg/L and a 96h-NOEC of 38 µg/L. Study was considered valid and acceptable in the RAR. The results can be used for classification purposes.

Concluding

From the above summaries it can be concluded that there are three reliable effect concentrations for three aquatic invertebrate species, i.e. a 48h-EC_{50} of 0.40 mg/L for *Daphnia magna*, a 96h-EC_{50} of 0.092 mg/L for *Crassostrea virginica*, and a 96h-EC_{50} of 0.065 mg/L for *Mysidopsis bahia*. The EC_{50} value of 0.065 mg/L from the mysid study is the lowest available value, and that study is considered the key study for classification purposes.

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

There are two studies with aquatic primary producers available in the RAR of which one investigated growth inhibition of pyriproxyfen to algae and one to aquatic plants.

Blasberg, Hicks and Cramer (1991) performed a 72-hours static algal growth inhibition test with pyriproxyfen (purity of 97.2%) using the green algae *Raphidocelis subcapitata* (synonyms: *Pseudokirchneriella subcapitata*; *Selenastrum capricornutum*) according to OECD TG 201 (CA 8.2.6.1/01; RAR B.9.2.6.1). Five concentrations were tested with the nominal test concentrations being 0.025, 0.05, 0.10, 0.20 and 0.40 mg/L. Control and solvent control (0.1 mL/L acetone) were included. Each treatment was conducted in triplicate. Test vessels were 250-mL glass Erlenmeyer flasks containing 100 mL synthetic medium. Initial cell density was 1.4 x 10^4 cells/mL. Growth was daily assessed. Flask were incubated under continuous light and shaking. Analytical monitoring (GC/LC) was performed at test start and end. Temperature ranged 23-24 °C and pH ranged 7.2-8.4. The actual pyriproxyfen concentrations ranged 69-83% of nominal test concentrations. The mean measured test concentrations were 0.020, 0.038, 0.069, 0.15 and 0.33 mg/L. Growth was exponential in the control (107 fold increase after 72 h). Control and solvent control did not differ significantly. A significant inhibitory effect compared to pooled control was observed for the 0.038, 0.069, 0.15 and 0.33 mg/L treatments.

A 72-h EC_{50} of 0.064 mg/L and a 72h-NOEC of 0.020 mg/L was reported by the applicant. In the RAR raw data was analysed according to OECD TG 201, and the following effect concentrations were reported: 72h-E_bC_{50} of 0.064 mg/L and a 72-h E_rC_{50} of 0.111 mg/L based on mean measured concentrations. It was also indicated that the validity criteria were met. Study was considered valid and acceptable in the RAR. The dossier submitter notes that EC_{10} values were not reported, and as raw data is not available these could not be derived. The results can be used for classification purposes.

Hoberg (1996) performed a 14-day semi-static Lemna growth inhibition test with pyriproxyfen (purity of 98.4%) using duckweed (*Lemna gibba*) according to FIFRA guidelines 122-2 and 123-2, US EPA (1982) (CA 8.2.7/01; RAR B.9.2.7.1). Five concentrations were tested with the nominal test concentrations being 0.023, 0.045, 0.090, 0.18 and 0.36 mg/L. Control and solvent control (0.1 mL/L acetone) were included. Medium was Hoagland's medium. Test volume and test vessels were not specified. Test was performed in triplicate. Each replicate contained five plants with three fronds. Fronds were counted and inspected every three days. Following the observations on days 3, 6, 9 and 12, the fronds were transferred to appropriate freshly prepared test or control solutions. At test termination (day 14), the fronds for each replicate were counted and their weight was determined. Temperature was measured continuously, pH at test start and after 3, 6, 9 and 12 days in new and aged solutions. Analytical monitoring was performed at day 6 in newly prepared solution and at day 9 in three 3d-old. Temperature ranged 24-25 °C, pH ranged 4.5-6.2, light intensity ranged generally 4300-5600 lux. The actual pyriproxyfen concentrations averaged 61-102% and 24-31% of nominal test concentrations at day 6 (newly prepared solution) and day 9 (3-old solution), respectively. The mean measured test concentrations were 0.016, 0.026, 0.049, 0.078 and 0.18 mg/L. At test end, the average (± st.dev) number of fronds per replicate were 392±88, 357±33, 358±69, 500±68, 424±46, 398±35, and 380±36 in the control, solvent control, 0.016, 0.026, 0.049, 0.078 and 0.18 mg/L treatments, respectively. Control and solvent control did not differ significantly. There was no significant reduction in frond production compared to the pooled control in any of the test concentrations with % reduction amounting to 4.4, -33, -13, -6 and -1.6%, respectively. At test end, the frond biomass averaged (± st.dev.) 0.1185 ± 0.0337, 0.1190 ± 0.0287, 0.1176 ± 0.0572, 0.1302 ± 0.0194, 0.1252 ± 0.0122, 0.1140 ± 0.0242 and 0.1147 ± 0.0130 in the control, solvent control, 0.016, 0.026, 0.049, 0.078 and 0.18 mg/L treatments, respectively. There was no significant reduction in frond biomass (dry weight) compared to the pooled control in any of the test concentrations with % reduction amounting to 1.0, -9.6, -5.4, 4.0 and 3.5%, respectively. The 14d-EC_{50} based on frond density and biomass, was estimated to be >0.18 mg/L based on mean measured test concentrations. The corresponding NOEC was determined to be 0.18 mg/L, the highest concentration tested. The study was considered acceptable in the RAR. The dossier submitter notes that the test duration is with 14 days twice as long as specified by OECD TG 221, i.e. 7 days. However, as indicated in section I.2.3.2 of the CLP guidance exposure up to 14 days is considered acceptable. The mean measured test concentrations are based on two measurements only, i.e. one fresh and one aged solution, while during the study four renewals took place. This yields some uncertainty to the true exposure levels. The NOEC for classification purposes is ≥0.18 mg/L, as no significant effects were observed at the highest treatment.

Concluding

From the above summaries it can be concluded that there is one reliable algal inhibition study yielding an 72h-E_rC_{50} of 0.111 mg/L and a 72h-NOEC of 0.020 mg/L (mean measured) for the green algae *Raphidocelis subcapitata*, and one Lemna growth inhibition study yielding an 14d-EC_{50} of >0.18 mg/L and a 14d-NOEC of ≥0.18 mg/L (mean measured) for the duckweed *Lemna gibba*. The lowest values reported for the green algae will be used for classification purposes.

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

No acute toxicity data are available for other aquatic organisms.

11.6 Long-term aquatic hazard

The results presented in the table below are from the studies conducted with pyriproxyfen, and critical studies performed with two degradation products (PYPAC and 4'-OH-Pyr). The RAR also reports studies conducted with the formulation Pyriproxyfen 10EC (S-71639) that are not further discussed as explained in section 11.5. Summaries are provided in the subsequent sections. For further details, please see Annex 1 to this CLH proposal (section 4.3.1).

Table 73: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
Fish					
Fish early life stage toxicity test flow-through US EPA 72-4; GLP	Rainbow trout (*Oncorhynchus mykiss*)	Technical pyriproxyfen Purity: 97.2%	95d- NOEC = 0.0043 mg/L (mean measured, based on reduced mean standard length and mean wet weight) EC_{10} = 0.012 mg/L (mean measured)	NOEC preferred, as EC_{10} based on bad fit. Acceptable	Anonymous (1991) CA 8.2.2.1/01; NNW-11-0062; RAR B.9.2.2.2 Anonymous (2016) CA 8.2.2.1/01b; NNW-0248; RAR B.9.2.2.3
Fish full life cycle (FFLC) test flow-through FFLC TG, Japanese ministry of the environment (Annex 6-2); GLP	Medaka (*Oryzias latipes*)	Technical pyriproxyfen Purity: 98.7%	189d-NOEC = 0.0027 mg/L (mean measured, based on overall hatchability of F1 generation)	EC_{10} value could not be calculated Acceptable Key study	Anonymous (2007) CA 8.2.2.2/01; NNW-0181; RAR B.9.2.2.4
Amphibian					
Amphibian metamorphosis assay flow-through US OPPTS 890.1100, OECD TG 231; GLP	African clawed frog (*Xenopus laevis*)	Technical pyriproxyfen Purity: 99.5%	21d-NOEC = 0.0017 mg/L (mean measured, based on reduced hindlimb length)	EC_{10} value could not be calculated Acceptable Key study	Anonymous (2012) CA 8.1.4/01; NNW-0211; RAR B.9.1.4.1
Aquatic invertebrates					
Freshwater					

crustaceans					
Daphnia reproduction study flow-through US EPA 72-4; GLP	Water flea (*Daphnia magna*)	[pyridyl-2,6-^{14}C]-pyriproxyfen, Radiochemical purity: 100%	21d NOEC = 0.000015 mg /L <u>Combined fit</u> **21d-EC_{10} = 0.0000088 mg/L** (mean measured, based upon effects on reproduction)	Acceptable **Key study**	Blakemore et al. (1992) CA 8.2.5.1/01; NNW-21-0075 G. Lewis (2016); CA 8.2.5.1/01b; NNW-0247; RAR B.8.2.5.1
Daphnia reproduction study semi-static non-guideline, but complying to OECD TG 211;non-GLP	Water flea (*Daphnia pulex)*	Technical pyriproxyfen Purity: 97.2%	21d-NOEC = 0.00001 mg /L (nominal)	No analytical monitoring; NOEC based on body weight. Reproduction might be more sensitive endpoint. Due to lack of individual data on reproduction and methods of statistical analysis, the results on reproduction could not be evaluated. Not acceptable	Kagoshima et al. (1995) CA 8.2.5.2/05; NNW-50-0120; RAR B.9.2.5.10
Daphnia reproduction study flow-through US EPA 72-4; GLP	Water flea (*Daphnia magna*)	PYPAC Purity: 99.1% --- 4'-OH-Pyr Purity: 100%	21d-NOEC = 0.00474 mg/L (mean measured, reduction in reproduction) --- 21d-NOEC = <0.00439 mg/L (mean measured, reduction in reproduction)	Study with degradation products Acceptable **Supporting study**	Rhodes et al. (1996) CA 8.2.5.1/02; NNW-0127; B.9.2.5.2
Daphnia reproduction study flow-through US EPA 72-4; GLP	Water flea (*Daphnia magna*)	4'-OH-Pyr Purity: 99.2%	21d-NOEC = 0.0029 mg/L (mean measured, reduction in reproduction)	Study with degradation product Acceptable **Supporting study**	Shaw (2015a) CA 8.2.5.1/03; NNW-0238; B.9.2.5.3
Freshwater invertebrate	Freshwater amphipod	Technical pyriproxyfen,	42d-NOEC = 0.041 mg/L	Concentration expressed as pore	Picard (2015)

chronic toxicity static-renewal US EPA OCSPP 850.1770; GLP	(*Hyalella azteca*)	Purity: 99.2%	(mean measured, based on effects on mean length)	water. Acceptable	CA 8.2.5.4/01; NNW-0243; RAR B.9.2.5.14
Freshwater crustacean growth study semi-static non-guideline; non-GLP	*Asellus hilgendorfii*	Technical pyriproxyfen Purity: 97.2%	21d-NOEC = ≥0.01 mg/L (nominal)	No analytical monitoring; Limited reporting; No replicates Not acceptable	Hagino and Matsuda (1992a) CA 8.2.5.2/01; NNW-21-0081; RAR B.9.2.5.6
Freshwater crustacean reproduction study semi-static non-guideline; non-GLP	*Asellus hilgendorfii*	Technical pyriproxyfen Purity: 97.2%	19d- to 24d-NOEC = ≥0.01 mg/L (nominal)	No analytical monitoring; Limited reporting Not acceptable	Hagino an Matsuda (1992d) CA 8.2.5.2/02; NNW-21-0082; RAR B.9.2.5.7
Marine crustaceans					
Mysid life-cycle test flow-through US EPA 72-4; ASTM E1191 – 87; GLP	Mysid (*Mysidopsis bahia*)	Technical pyriproxyfen Purity 95.3%	28d-NOEC = 0.00081 mg/L (mean measured, based on reproductive success) 28d-EC_{10} = 0.00087 mg/L (mean measured)	Based on reproductive success (differently defined than TG). EC_{10} unreliable due to large CI. Acceptable (with limitations)	Machado (1995); CA 8.2.5.2/06 NNW-51-0119 G. Lewis (2016); NNW-0249; RAR B.9.2.5.11
Marine crustacean growth study semi-static non-guideline; non-GLP	*Tigriopus japonicus*	Technical pyriproxyfen Purity: 97.2%	5d-NOEC = ≥0.01 mg /L (nominal)	No analytical monitoring; Limited reporting; No replicates at test start Not acceptable	Hagino an Matsuda (1992c) CA 8.2.5.2/03; NNW-21-0085; RAR B.9.2.5.8
Marine crustacean reproduction study	*Tigriopus japonicus*	Technical pyriproxyfen	8d-NOEC = ≥0.01 mg /L (nominal)	No analytical monitoring; Limited reporting; No	Hagino an Matsuda (1992b) CA 8.2.5.2/04; NNW-21-0084; RAR B.9.2.5.9

semi-static non-guideline; non-GLP		Purity: 97.2%		replicates at test start Not acceptable	
Freshwater insects					
Sediment-water chironomid toxicity test (spiked water) Static OECD TG 219; GLP	Midge (*Chironomus riparius*)	[pyridyl-2,6-^{14}C]-pyriproxyfen, Radiochemical purity: 99.6%	28d-NOEC = 0.031 mg/L (mean measured, based on emergence) 28d-NOEC = 0.010 mg/L (nominal, based on reduction of development rates)	Concentration expressed as overlaying water. Not possible to derive relevant and potentially lower pore water concentrations. EC_{10} not reliable, as confidence limits could not be determined Acceptable (with restrictions)	Putt (2003) CA 8.2.5.3/01; NNW-0157; RAR B.9.2.5.13
Mayfly toxicity test semi-static non-guideline; GLP	Mafly (*Cloeon dipterum*)	[phenoxyphenyl-^{14}C]-pyriproxyfen Radiochemical purity: 100%	30d-NOEC = ≥0.000196 mg/L (mean measured)	Developmental stage nymphs at test initiation unknown Unreliable	Biester (2011) CA 8.2.5.2/07; NNW-0209; RAR B.9.2.5.12
Aquatic insect toxicity tests semi-static non-guideline; GLP	Mayfly (*Cloeon dipterum*) Water bug (*Plea minutissima*) Damselfly (*Coenagrion pulchellum* & *Enallagma cyanthigerum*)	Pyriproxyfen Purity: 99.2% / 100%	*C. dipterum* 28d-NOEC = 0.0167 mg/L (time weight average, based on mortality) 28d-EC_{10} = 0.0147 mg/L (time weight average, based on mortality) *P. minutissim* 28d-NOEC = ≥0.0864 mg/L *C. pulchellum* & *E. cyanthigerum* 28d-NOEC = 0.0006 mg/L 28d-EC_{10} = 0.00021 mg/L	*C. dipterum* and *P. minutissim* Acceptable (with restrictions) *C. pulchellum* & *E. cyanthigerum* Unreliable	Roessink (2018) CA 8.2.5.2/08; NNW-0259; RAR B.9.2.5.15

			(mean measured)		
Algae or other aquatic plants					
Algal growth inhibition static OECD 201; GLP	Green algae (*Pseudokirchneriella subcapitata*)	Technical pyriproxyfen Purity: 97.2%	72h-NOEC = 0.02 mg/L (mean measured, inhibition of absolute cell numbers) 72h-E_rC_{50} = 0.111 mg/L (mean measured)	Acceptable Key study	Blasberg, Hicks and Cramer (1991) CA 8.2.6.1/01; NNW-11-0068; RAR B.9.2.6.1
Lemna growth inhibition semi-static US EPA 122-2 and 123-2; GLP	Duckweed (*Lemna gibba*)	Technical pyriproxyfen Purity: 98.4%	14d-NOEC = ≥0.18 mg/L (mean measured) 14-day EC_{50} >0.18 mg /L (frond density)	Acceptable	Hoberg (1996) CA 8.2.7/01; NNW-0126; RAR B.9.2.7.1

11.6.1 Chronic toxicity to fish

There are two studies available in the RAR that investigated the long-term toxicity of pyriproxyfen to fish.

Anonymous (1991) performed a GLP-compliant 95-day fish early life stage toxicity test under flow-through conditions with pyriproxyfen (purity of 97.2%) using rainbow trout (*Oncorhynchus mykiss*) according to US EPA-FIFRA 72-4, and in agreement with OECD TG 210 (2013 version) (CA 8.2.2.1/01; RAR B.9.2.2.2). Five concentrations were tested with the nominal test concentrations being 0.0019, 0.0038, 0.0075, 0.015 and 0.030 mg/L. Control and solvent control (dimethylformamide; DMF) were included. Medium was soft blended well water (total hardness of 162-190 mg/L as $CaCO_3$). Flow rate was 98 L/replicate/day (134 L/replicate/day during last 2 weeks). All treatments, including controls, were tested in quadruplicate and contained 35 newly fertilised eggs placed in an incubator cup (total of 140 eggs/treatment). An additional 50 eggs were placed into each of 4 control aquaria to assess fertilisation success on day 12. When hatching commenced, the number of embryos hatched in each incubation cup was recorded daily until hatch was completed (day 36). Feeding was commenced on day 48 (14 days post-hatch (dph)) when the sac-fry began to exhibit normal swim-up behaviour. Abnormal behavioural, normal swim-up behaviour, abnormal physical change and mortality were monitored daily. Growth was determined on 35 and 61 dph using respectively the photographic method and by sacrificing and measuring length and weight of fish. Test concentrations were analysed (GC-LC) and temperature, dissolved oxygen, conductivity and pH were measured on days 0, 1 and 7 and weekly thereafter. Temperature ranged 9.8-11.3 °C, pH ranged 8.0-8.6 and dissolved oxygen ranged 6.8-10.6 mg/L (64 and 98% saturation, respectively). From day 84 onward, oxygen concentrations <75% saturation were periodically found in all treatments, except for the control. This was not considered to have impacted the validity of the data, and likely reflected the presence of DMF as vehicle to dissolve the test compound. The mean measured concentrations were: 0.0018, 0.0043, 0.0067, 0.014 and 0.026 mg/L, corresponding to 87-113% of nominal test concentrations. Egg viability averaged over all treatments 98%. Hatching averaged 99, 96, 100, 99, 98, 99 and 98% in the control, solvent control, 0.0018, 0.0043, 0.0067, 0.014 and 0.026 mg/L treatments, respectively. At 61-d post-hatch survival averaged 97, 97, 92, 98, 90, 83, and 98%; standard length averaged 48.6, 48.4, 48.5, 48.0, 48.2, 47.1, 45.7 and 45.1 mm; and wet weight 1.71, 1.71, 1.71, 1.63, 1.67, 1.60, 1.43 and 1.35 g, respectively. Control and solvent control did not differ significantly with respect to any of the endpoints, and were pooled for analysis. Fry survival was significantly impacted at 35 and 61 dph in the 0.014 mg/L treatment only (single deviating replicate). Standard length and wet weight were significantly reduced in the 0.0067,

0.014 and 0.026 mg/L treatments on 61 dph with the reduction in length being 2.8, 5.7 and 7.1% respectively, and the reduction in wet weight being 6.2, 16.2 and 21.0%, respectively. Swim-up began at approximately the same time throughout all test concentrations and appeared to proceed at the same rate in all test concentrations. Compound-related physical and behavioural effects that were noted during this study included the following: 1) fish resting on the bottom of the test chamber, 2) quiescence, 3) discoloration, 4) spinal curvature, and 5) irregular respiration. These effects were noted primarily in the 0.0067, 0.014, and 0.026 mg/L treatments, and they had effectively disappeared by day 77 (43-Day post-hatch). The NOEC for length and wet weight was set at 0.0043 mg/L based on mean measured concentrations of pyriproxyfen. The level of effect was 2.8 and 6.2% reduction, respectively. Anonymous (2016) used the data from this study to calculate EC_{10} values of 0.012 and 0.037 mg/L for wet weight and length, respectively. In the RAR the EC_{10} values were assessed according to EFSA Supporting publication 2015: EN-924, and it was concluded that the EC_{10} based on wet weight is "bad" based on its normalised width (NW) of the confidence limit ((upper limit – lower limit) / median estimate), and the EC_{10} based on standard length as poor based on its NW. The NOEC was preferred above the EC_{10} values in the RAR. Study was considered acceptable in the RAR and can be used for classification purposes.

Anonymous (2007) performed a GLP-compliant 189-day full life cycle toxicity test with medaka (*Oryzias latipes*) under flow-through conditions with pyriproxyfen (purity of 98.7%) according to the medaka (*Oryzias latipes*) full life cycle test guideline (Ministry of the Environment, Japan, Annex, 6-2, November 2002) (CA 8.2.2.2/01; RAR B.9.2.2.4). Three concentrations were tested with the nominal test concentrations being 1.0, 3.2 and 10 µg/L. Control and solvent control (not specified) were included. Medium and test vessels type were not specified. Flow rate was 13 volumes/day. Numbers of fish tested in the P and F1-generation were 4 replicates of 15 organisms per test concentration. In the P-generation (fertilised egg to adult stage at 60 dph) 20 fish per treatment were investigated for total length, wet weight, condition factor, deformities, genetic sex (colouration of the body and the outer rays of the caudal fin), secondary sex characteristics (fin morphology; tl = total length of individual fish; dm = maximum length of dorsal fin; dc = cleft depth between the last ray and preceding one of dorsal fin; am = maximum length of anal fin; a2 = length of the second ray from the last one of the anal fin), gonad weight, gonadosomatic index (GSI), liver weight, hepatosomatic index (HIS), hepatic vitellogenin (VTG) concentration, and functional sex (histology of gonads, ovo-testis). For the reproduction phase, 8 pairs (1 male and 1 female) per test concentration were impartially selected. On 114 dph, i.e., after the reproduction phase, the parameters determined on 60 dph were determined from all surviving fish from the P-generation. In the F1-generation, eggs which were collected prior to termination of the P-generation (99-101 dph of the P-generation) were exposed to the chemical for 60 dph, and the effects on the parameters listed for day 60 of the P-generation were investigated. Test concentrations were analysed (GC) and temperature, dissolved oxygen, and pH were measured weekly. Water hardness and alkalinity was determined in the control and highest treatment at test start and end, and conductivity at test end. Temperature ranged 23.9-26.1 °C, pH ranged 6.68-8.95, dissolved oxygen ranged 5.16-9.12 mg/L (66-117% saturation, respectively), hardness ranged 30-80 mg/L, alkalinity ranged 383 -392 mg/L, and conductivity was 845-850 µS/cm at test end. The mean measured concentrations were 0.84, 2.7 and 8.6 µg/L. The results determined in the P-generation up to 60 dph, as well as the endpoints determined in the P-generation at 61-114 dph and the F1-generation at 60 dph are summarized in the tables below. These tables are taken from the RAR (but originate from the original study report). Treatments were compared to pooled control, unless when control and solvent control differed significantly, then comparison was made to the solvent control. Statistically significant effects are indicated in the table below with an asterisks. Below the outcomes are discussed.

In the P-generation (up to 60 dph), there were no statistically significant effects in: hatchability; time of hatching; behavioural changes or deformities; survival; total length of females; body weight of males and females; gonad weight of females; GSI for females; fin morphology for females (dm/tl; dc/dm; am/tl; a2/tl); fin morphology for males (dm/tl; am/tl; a2/tl); liver weight of males; HIS for males; and hepatic VTG concentration in females. While one of the treatments differed significantly compared to the pooled/ solvent control for the following endpoints, this was not considered treatment related as there was no dose-response relationship: total length of males; gonad weight of males; GSI for males; fin morphology for males (dc/dm); liver weight of females; and HIS for females. The hepatic VTG concentrations in males differed statistically form the pooled control in the 0.84 and 8.6 µg/L treatments, this was not considered treatment related as it was considered to be caused by unusual low control and solvent control hepatic vitellogenin concentrations.

Overall, in the P-generation (up to 60 dph) no significant and treatment related effects of pyriproxyfen were observed.

In the P-generation (61-114 dph), there were no statistically significant effects in: number of eggs per female and for both sexes: survival; total length; body weight; condition factor; gonad weight; GSI; fin morphology (dm/tl; dc/dm; am/tl; a2/tl); liver weight; HSI: hepatic VTG concentration. All males showed papillary processes on the anal fin while none of the females showed these processes. Only for fertility one treatment showed a significant decrease compared to the pooled control but this was not considered treatment related, as there was no dose response. Overall, in the P-generation (61-114 dph) no significant and treatment related effects of pyriproxyfen were observed.

In the F1-generation, there were no statistically significant effects in: survival; behavioural changes or deformities; total length of females; HIS for males; and for both sexes: body weight; condition factors; gonad weight; GSI; fin morphology (dm/tl; dc/dm; am/tl; a2/tl); liver weight; and hepatic VTG concentration. While hatchability at 99 dph was significantly decreased in the highest treatment compared to the solvent control, this was not considered to be treatment related as apparently hatchability at 100 and 101 dph did not differ significantly from the control. Other endpoints only significantly affected at one test concentration were total length for males; HIS for females. Except for two male fish (one at 0.84 and one at 8.6 µg test item/L), all males showed papillary processes on the anal fin while none of the females showed these processes. Overall, in the F1-generation no significant and treatment related effects of pyriproxyfen were observed.

No test item related sex-reversal occurred in P or F1-generations as determined by colouration and verified by histopathological analysis, The applicant concluded that no pyriproxyfen related effects were found, and determined the NOEC as ≥8.6 µg/L.

In the RAR the study was assessed according to OECD Review Paper No. 95 on fish life-cycle tests (2008), while also considering the recommendations in the draft OECD TG 240 for a multigeneration study with Medaka. It was noted that less fish were tested than recommended by OECD, i.e. each treatment consisted of 60 fish (15 in each quadruplicate) instead of recommended 100; in the reproduction phase 8 males and 8 females per treatment were assessed instead of 12 males and 20 females as recommended; the effects were assessed in 20 fish per treatment only. Also the applied time frames deviated from recommendations in OECD. Overall, these deviations were not expected to have affected the validity of the study, and as adequate parameters were assessed. In the RAR, the hatchability in the F1-generation was re-assessed. A statistical difference between control and solvent control was found, and, therefore, the analysis for the pyriproxyfen treatments was based on comparison with both controls separately. The overall hatchability in the F1-generation was statistically significantly reduced at the highest tested concentration (8.6 µg/L; mean measured) in both cases (by 8.6% from the untreated control and by 15.9% from the solvent-control). The NOEC was concluded to be 0.0027 mg/L. In the RAR it was reported that a reliable EC_{10} value could not be calculated. The study was considered acceptable in the RAR, and the results can be used for classification purposes.

Table B.9.2.2.4-01 Hatchability and time of hatching of P-generation medaka exposed to pyriproxyfen

Mean measured concentration (µg/L)	Hatchability (%)	Time of hatching (day)
Control	70	10.0
Solvent control	86	10.3
0.84	86	10.5
2.7	74	10.0
8.6	76	12.0

Table B.9.2.2.4-02 Summary of the parameters of P-generation determined at 60 days post-hatching

Mean measured conc. (µg/L)	Mean survival (%)	Sex ratio		Total length (mm)		Body weight (mg)		Condition factor		Gonad weight (mg)		GSI (%)	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Control	91.8	9	11	32.5	30.9	499	366	14.6	12.3	76.4	3.39	15.3	0.93
Solvent control	100	10	10	31.9	31.3	464	361	14.3	11.7	62.0	3.04	13.4	0.84
0.84	95.7	8	11	31.3	32.7*	454	403	14.8	11.6	67.2	4.05*	14.6	1.02
2.7	93.5	8	11	32.0	31.0	468	352	14.2	11.8	66.2	3.90	14.2	1.13*
8.6	93.8	10	10	31.6	30.8	433	350	13.7	11.9	71.9	3.42	15.9	1.00

* Statistically significant when compared to pooled controls ($p < 0.05$)

Table B.9.2.2.4-03 Summary of the fin morphology characteristics of P-generation determined at 60 days post-hatching

Mean measured concentration (µg/L)	dm/tl		dc/dm		am/tl		a2/tl		% appearance of small papillary processes on the posterior region of the anal fin	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Control	0.109	0.147	0.096	0.326	0.110	0.152	0.065	0.109	0	91
Solvent control	0.110	0.148	0.093	0.311	0.108	0.149	0.066	0.105	0	100
0.84	0.113	0.145	0.079	0.314	0.112	0.147	0.067	0.109	0	100
2.7	0.111	0.147	0.088	0.357*	0.111	0.149	0.069	0.109	0	100
8.6	0.111	0.151	0.082	0.312	0.106	0.150	0.067	0.110	0	100

* Statistically significant when compared to pooled controls ($p < 0.05$)

Table B.9.2.2.4-04 Mean liver weights, hepatosomatic indices and hepatic vitellogenin concentrations of P-generation determined at 60 days post-hatching

Mean measured concentration (µg/L)	Liver weight (mg)		HSI (%)		Hepatic VTG (ng/mg)	
	♀	♂	♀	♂	♀	♂
Control	29.2	12.5	5.9	3.3	1202	0.17
Solvent control	25.1	12.0	5.4	3.3	907	0.27
0.84	19.2*	10.8	4.2*	2.7	778	0.54*
2.7	25.7	10.2	5.4	2.9	1012	0.57
8.6	21.4	10.6	4.9	3.1	556	0.65*

* Statistically significant when compared to pooled controls ($p < 0.05$)

Table B.9.2.2.4-05 Histopathological evaluation of P-generation Medaka determined at 60 days post-hatching

Mean measured concentration (µg/L)	Number of fish		Ovotestis		Other male deformities		Non-functional gonad	
	♀	♂	♀	♂	♀	♂	♀	♂
Control	9	11	N	N	N	N	N	N
Solvent control	10	10	N	N	N	N	N	N
0.84	9	11	N	N	N	N	Immature ovary: 1	N
2.7	8	11	N	N	N	N	N	N
8.6	10	10	N	1Y[a]	N	N	Immature ovary: 1	N

[a] One male had ovotestis

N: none

Y: yes

Table B.9.2.2.4-06 Mean fecundity and fertility of P-generation during the reproduction phase

Mean measured concentration (µg/L)	Mean fecundity (eggs/female/day)	Mean fertility
Control	35.0 ± 7.8	89.4 ± 8.9
Solvent control	35.9 ± 10.8	94.3 ± 8.8
0.84	38.2 ± 8.4	96.0 ± 1.4
2.7	39.2 ± 15.1	89.8 ± 11.1**
8.6	34.2 ± 7.2	91.3 ± 9.9

** Statistically significant when compared to the solvent control ($p < 0.05$)

Table B.9.2.2.4-07 Summary of the parameters of P-generation determined at 61-114 days post-hatching

Mean measured concentration (µg/L)	Mean survival on day 114 (%)	Sex ratio		Total length (mm)		Body weight (mg)		Condition factor		Gonad weight (mg)		GSI (%)	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Control	100	8	8	37.6	36.1	775	508	14.4	10.8	105.3	4.99	13.4	0.98
Solvent control	100	8	8	37.5	36.6	743	549	14.0	11.3	107.4	5.59	14.4	1.03
0.84	100	8	8	37.5	37.3	735	554	13.9	10.7	98.9	5.38	13.4	0.98
2.7	87.5	6	8	36.8	36.9	706	538	14.2	10.6	97.3	4.68	13.6	0.86
8.6	93.8	7	8	36.5	35.9	648	531	13.3	11.3	91.1	4.55	13.8	0.84

Table B.9.2.2.4-08 Summary of the fin morphology characteristics of P-generation determined at 61-114 days post-hatching

Mean measured concentration (µg/L)	dm/tl		dc/dm		am/tl		a2/tl		% appearance of small papillary processes on the posterior region of the anal fin	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Control	0.115	0.139	0.056	0.363	0.104	0.141	0.069	0.102	0	100
Solvent control	0.117	0.145	0.055	0.349	0.108	0.146	0.068	0.114	0	100
0.84	0.119	0.141	0.048	0.339	0.108	0.144	0.068	0.109	0	100
2.7	0.116	0.138	0.058	0.362	0.106	0.144	0.066	0.113	0	100
8.6	0.113	0.141	0.056	0.362	0.107	0.147	0.068	0.112	0	100

Table B.9.2.2.4-09 Mean liver weights, hepatosomatic indices and hepatic vitellogenin concentrations of P-generation determined at 61-114 days post-hatching

Mean measured concentration (µg/L)	Liver weight (mg)		HSI (%)		Hepatic VTG (ng/mg)	
	♀	♂	♀	♂	♀	♂
Control	41.1	9.2	5.4	1.8	1310	0.41
Solvent control	39.1	9.9	5.4	1.8	767	0.21
0.84	42.5	9.7	5.8	1.8	1043	0.13
2.7	55.6	9.4	7.8	1.7	772	0.19
8.6	34.8	9.1	5.3	1.7	723	0.11

Table B.9.2.2.4-10 Histopathological evaluation of P-generation Medaka determined at 114 days post-hatching

Mean measured concentration (µg/L)	Number of fish		Ovotestis		Other male deformities		Non-functional gonad	
	♀	♂	♀	♂	♀	♂	♀	♂
Control	8	8	N	N	N	N	N	N
Solvent control	8	8	N	1Y[a]	N	N	N	N
0.84	8	8	N	N	N	N	N	N
2.7	6	8	N	N	N	N	N	N
8.6	7	8	N	N	N	N	N	N

[a] One male had ovotestis

N: none

Y: yes

Table B.9.2.2.4-11 Summary of the hatchability and time of hatching of F1 generation started on days 99, 100 and 101 and the pooled results for hatchability calculated by the RMS

Mean measured concentration (µg/L)	Hatchability (%)				Time of hatching (day)		
	99 dph	100 dph	101 dph	99-101 dph	99 dph	100 dph	101 dph
Control	76	88	83	82.08	9.5	9.5	10
Solvent control	94	86	88	89.17	9.8	9.3	9.8
0.84	90	91	88	89.58	9.8	9.8	10
2.7	94	94	93	93.33	9.8	9.5	9.8
8.6	71**	78	76	75.00*,**	9.5	10	10

* Statistically significant when compared to the control ($p < 0.05$)

** Statistically significant when compared to the solvent control ($p < 0.05$)

Table B.9.2.2.4-12 Summary of the parameters of F1-generation determined at 60 days post-hatching

Mean measured concentration (µg/L)	Mean survival (%)	Sex ratio		Total length (mm)		Body weight (mg)		Condition factor		Gonad weight (mg)		GSI (%)	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Control	93.3	21	35	28.9	28.2	297	257	12.2	11.4	31.3	2.11	9.9	0.80
Solvent control	100	25	35	28.8	28.8	315	284	13.2	11.9	32.2	2.63	10.1	0.97
0.84	98.3	33	26	29.1	28.2	316	263	12.8	11.7	32.8	2.54	10.3	0.86
2.7	98.3	33	26	28.3	27.9*	305	259	13.4	11.8	34.9	2.78	10.8	1.04
8.6	96.7	32	26	28.2	28.0	302	261	13.5	11.7	31.5	2.84	10.3	1.01

* Statistically significant when compared to pooled controls ($p < 0.05$)

Table B.9.2.2.4-13 Summary of the fin morphology characteristics of F1-generation determined at 60 days post-hatching

Mean measured concentration (µg/L)	dm/tl		dc/dm		am/tl		a2/tl		% appearance of small papillary processes on the posterior region of the anal fin	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Control	0.115	0.152	0.106	0.306	0.113	0.147	0.068	0.109	0	100
Solvent control	0.115	0.151	0.065	0.270	0.112	0.153	0.068	0.112	0	100
0.84	0.109	0.146	0.069	0.259	0.109	0.151	0.068	0.106	0	90
2.7	0.115	0.144	0.063	0.251	0.111	0.142	0.069	0.112	0	100
8.6	0.109	0.153	0.067	0.258	0.107	0.151	0.069	0.107	0	89

Table B.9.2.2.4-14 Mean liver weights, hepatosomatic indices and hepatic vitellogenin concentrations of F1-generation determined at 61-114 days post-hatching

Mean measured concentration (µg/L)	Liver weight (mg)		HSI (%)		Hepatic VTG (ng/mg)	
	♀	♂	♀	♂	♀	♂
Control	18.5	7.2	5.8	2.8	482	0.57
Solvent control	12.3	7.6	3.8	2.7	596	0.26
0.84	17.4	8.3	5.6**	2.8	765	0.44
2.7	15.2	7.8	4.7	2.9	520	0.88
8.6	14.3	8.2	4.7	2.9	603	0.65

Table B.9.2.2.4-15 Histopathological evaluation of F1-generation Medaka determined at 60 days post-hatching

Mean measured concentration (µg/L)	Number of fish		Ovotestis		Other male deformities		Non-functional gonad	
	♀	♂	♀	♂	♀	♂	♀	♂
Control	10	10	N	N	N	N	N	N
Solvent control	11	9	N	N	N	N	N	Immature testis: 1
0.84	10	10	N	N	N	N	Immature ovary: 1	N
2.7	10	10	N	N	N	N	N	N
8.6	11	9	N	N	N	N	N	N

* One male had ovotestis

N: none

Y: yes

Concluding

From the above summaries it can be concluded that there are two reliable chronic effect concentrations for two fish species, i.e. a 95d-NOEC of 0.0043 mg/L from a fish early life stage test with rainbow trout and a 189d-NOEC of 0.0027 mg/L from a fish full life cycle test with medaka. For neither studies could reliable EC_{10} values be derived. The lowest value obtained for medaka will be used for classification purposes.

11.6.2 Chronic toxicity to aquatic invertebrates

There are eleven studies available in the RAR that investigated the long-term toxicity of pyriproxyfen to aquatic invertebrates.

Freshwater crustaceans

Blakemore (1992) performed a GLP-compliant 21-day daphnia reproduction study under flow-through conditions with pyriproxyfen (purity of 97.2%) using *Daphnia magna* according to U.S. EPA Pesticide Assessment Guidelines, 72-4(b) (CA 8.2.5.1/01a; RAR B.9.2.5.1). The data were reanalysed by Lewis (2016) (CA 8.2.5.1/01b). A 17-day range finding study was performed. Two definitive test were reported both consisting of a control, solvent control (dimethylformamide; DMF) and five test concentrations. The nominal test concentrations were 2.4, 4.8, 10, 20 and 40 ng/L in the 1st test, and 18, 36, 75, 150 and 300 ng/L in the 2nd test. All treatments were conducted in quadruplicate. Each replicate consisted of 10 daphnid (<24h) incubated in a 1-L test vessel. Medium was a mixture of blended well water and reverse osmosis water with a total hardness of between 160 to 180 mg/L (as $CaCO_3$). Flow rate was sufficient for 5 exchanges per day (3.4-3.5 mL/chamber/minute). Feeding was twice daily (mixture of algae, yeast and fish food). Parent mortality, abnormal effects and first-brood were observed daily. Reproduction was observed three times a week. Parent length was determined at test end. Water quality (temperature, dissolved oxygen and pH) was determined in the low, middle and high treatments at days 0, 4, 7, 14 and 21, while analytical measurements (LSC) were conducted in composite samples taken from the four replicates on the same days. Water quality of the dilution water was determined daily. Temperature ranged 19-20 °C, pH ranged 8.2-8.4, dissolved oxygen ranged 7.8-8.5 mg/L (90 and 98% saturation, respectively). The mean measured concentrations were: 1.8, 4.4, 7.1, 15 and 31 ng/L in the 1st test, and 20, 27, 56, 120 and 240 ng/L in the 2nd test, respectively. This corresponds to 71-92%, respectively, 75-111% of nominal test concentrations. The results are presented in the tables below. There were no significant differences between control and solvent control in either test with respect to the investigated endpoints, i.e. survival, parent length, live young per adult and time to first brood. Treatments were therefore compared to the pooled controls in both tests. In the 1st test no significant effect were observed for any of the treatments compared to the pooled control even at the highest test concentration of 31 ng/L (see table below). In the second study, no significant effect on survival were observed for any of the treatments. The endpoints 'parent length' and 'young/adult reproduction days' were significantly decreased in all treatments when compared to the pooled control, and to the four highest treatments when compared to the solvent control only. Time to first brood was significantly delayed in the three highest test concentrations when compared to the pooled control. No comparison was made to the solvent control only. The applicant reported a 21d-NOEC of 15 ng/L (based on mortality, sublethal effects reduced reproductive success seen at test concentrations of 27 ng/L and above) and a 21d-NOEC of 20 ng/L (based on no significant compound-related effects). In a subsequent analysis the applicant reported an EC_{10} of 76 ng/L for adult length, and an EC_{10} of 8.8 ng/L for reproduction (number of young), both expressed based on mean measured test concentrations. In the RAR it was concluded that the slight but significant effects observed in the 2nd test are not due to the use of the solvent (DMF), as a downward trend over the entire concentration range tested was observed for both parent length and live young per adult. Therefore, comparison to merely solvent control is not justified. The 21d-NOEC was concluded to be 15 ng/L (based on parent length and live young per adult). The 21d-LOEC was determined to be 20 ng/L. The 21d-EC_{10} of 8.8 ng/L was used in the RAR, but it was noted that ECx derivation was not repeated by the RMS. The study was considered acceptable. The dossier submitter agrees with the comment in the RAR that slight differences in outcome of similar test are not uncommon, even when performed under highly similar conditions (same strain, same lab, etc.). Still the results from the two tests should be analysed separately, with the 1st test yielding a 21d-NOEC of ≥31 ng/L (as no significant effects were observed), and the 2nd test yielding a 21d-NOEC of <20 ng/L (as significant and relevant effects were observed in all treatments with respect to parent length and live young per adult). The dossier submitter fitted a log-logistic model to the available reproduction summary data from the 2nd test as presented in the table below and obtained a slightly higher 21d-EC_{10} of 12.3 ng/L (see figure below for the model fit). Considering, that the applicant had the availability of the raw data and that both values are close to each other, the 21d-EC_{10} of 8.8 ng/L can be used for classification purposes.

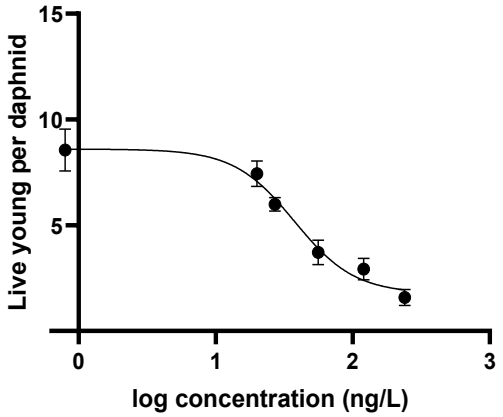

Figure 2. Fitting of log-logistic model on daphnid reproduction data from 2nd test (based on summary data (average ± deviation) from Blakemore (1992) (CA 8.2.5.1/01a; RAR B.9.2.5.1), as calculated by the Dossier Submitter.

Table B.9.2.5.1-01 Percent survival, behavioural observations, adult Daphnid length, young/adult reproduction days and time to first brood of *Daphnia magna* continuously exposure to ^{14}C-pyriproxyfen for 21 Days

Mean measured test concentration (mg a.s./L)	Day-21 Adult Survival[a] (%)	Day-21 Observation[d]	Day-21 Adult Daphnid Length[b] (mm)	Young/Adult Reprod. Days[b]	Time to First Brood[b]
Study #1					
Control	38 (97.5 ± 5.00)	---	3.91 ± 0.19	8.35 ± 0.35	8.00 ± 0.0
Solvent control	38 (95.0 ± 10.0)	2LD	3.91 ± 0.14	7.87 ± 0.74	7.00 ± 0.0
Pooled controls[c]	(96.3 ± 7.44)	---	3.91 ± 0.16	8.11 ± 0.60	7.50 ± 0.53
0.0000018	37 (92.5 ± 5.00)	---	3.99 ± 0.09	8.68 ± 0.30	7.00 ± 0.0
0.0000044	37 (92.5 ± 9.57)	---	3.89 ± 0.13	8.27 ± 0.34	7.50 ± 0.58
0.0000071	38 (95.0 ± 5.77)	1LD	3.96 ± 0.13	9.07 ± 0.68	8.00 ± 0.0
0.000015	38 (95.0 ± 5.77)	---	3.93 ± 0.10	9.12 ± 0.70	8.00 ± 0.0
0.000031	38 (95.0 ± 5.77)	1LD	3.81 ± 0.14	7.84 ± 0.74	8.00 ± 0.0

Mean measured test concentration (mg a.s./L)	Day-21 Adult Survival[a] (%)	Day-21 Observation[d]	Day-21 Adult Daphnid Length[b] (mm)	Young/Adult Reprod. Days[b]	Time to First Brood[b]
Study #2					
Control	40 (100.0 ± 0.00)	---	4.10 ± 0.13	9.02 ± 0.99	8.00 ± 0.0
Solvent control	40 (100.0 ± 0.00)	---	4.03 ± 0.16	8.09 ± 0.87	8.00 ± 0.0
Pooled controls[c]	(100.0 ± 0.00)	---	4.06 ± 0.15	8.55 ± 0.99	8.00 ± 0.0
0.000020	40 (100.0 ± 0.00)	---	3.93 ± 0.14**	7.43 ± 0.61**	8.00 ± 0.0
0.000027	40 (100.0 ± 0.00)	---	3.88 ± 0.12**/*	5.99 ± 0.32**/*	8.00 ± 0.0
0.000056	40 (100.0 ± 0.00)	---	3.67 ± 0.19**/*	3.72 ± 0.58**/*	8.8 ± 0.5**
0.000120	40 (100.0 ± 0.00)	1SM	3.54 ± 0.15**/*	2.93 ± 0.51**/*	10.8 ± 0.5**
0.000240	40 (100.0 ± 0.00)	40SM	3.33 ± 0.18**/*	1.58 ± 0.38**/*	11.0 ± 0.0**

a: Data were subjected to frequency analysis coupled with a one-tailed Fisher's exact test.

b: Data were subjected to a one-way analysis of variance (ANOVA) and Dunnett's multiple means comparison test.

c: Control and solvent control were compared by one-tailed Fisher's exact test or t-test. If significantly different, comparison was made with solvent control; otherwise, controls were pooled.

d: Unless otherwise indicated, the test water was clear and free of precipitate and all daphnids were normal in appearance and behaviour. The following abbreviations were used for observations: LD = Light Discoloration, SM = Smaller than Control

*: Denotes values significant different (P≤0.05) from the solvent control.

**: Denotes values significant different (P≤0.05) from the pooled controls.

Kagoshima et al. (1995) performed a non-GLP, non-guideline, 21-day daphnia reproduction study under semi-static conditions with pyriproxyfen (purity of 97.2%) using *Daphnia pulex*. (CA 8.2.5.2/05; RAR B.9.2.5.10). Three test were reported each consisting of five test concentrations with the nominal test concentrations being: 3.75, 7.5, 15, 30, 60 and 120 µg/L in test #1; 0.12, 0.24, 0.47, 0.94 and 1.88 µg/L in test #2; and 0.01, 0.03 and 0.06 µg/L in test #3. Controls were included in all tests, supplemented with a solvent control (DMSO + HCO-40, 1:1) in tests #1 and #2. All treatments were conducted in quadruplicate. Each replicate consisted of 10 daphnid (<24h). Test vessels, medium type, feeding, and renewal rate were not specified, neither was specified when observations were made, and when water quality parameters were measured. Analytical monitoring was not conducted. After 21 days, surviving individuals were transferred to fresh water to investigate the recovery during 21 days. The recovery part is not further discussed here. Temperature ranged 19-21°C dissolved oxygen was ≥80% of saturation and pH ranged 7.4 to 8.3. There were no treatment related effects on survival and behaviour reported, except at the highest test concentration in test #1, i.e. 120 µg/L where at day 4 mortality amounted to 97.5%. The time to first brood was significantly delayed at 0.24 µg/L and above. The number of live young per adult per reproduction day was significantly reduced at 0.06 µg/L and above. There was no effect on the number of exuvia at any test concentration. The adult daphnia length was significantly reduced at 0.03 µg/L and above. Based on reduced body length, a NOEC of 0.01 µg/L was reported. In the RAR it was concluded that the study is not acceptable, but can be used as supplementary information. The study generally complied with OECD TG 211 (erroneously referred to as OECD TG 202 in the RAR), but had several

deficiencies. Relevant details were missing on the study design, including medium renewal rate. and the statistical analysis applied. The raw reproduction data was not reported. Consequently it was not possible to evaluate the substantial, but according to the applicant insignificant, effects on reproduction that amounted to an offspring reduction of 13 and 16% in the 0.01 and 0.03 µg/L treatments, respectively. Furthermore, there was no analytical monitoring of the test concentrations. Considering above, the study is not used for classification purposes.

Picard (2015) performed a GLP-compliant 42-day sediment-water toxicity test under static-renewal conditions with pyriproxyfen (purity: 99.2%) using the freshwater amphipod *Hyalella azteca*, according to US EPA OCSPP 850.1770 (CA 8.2.5.4/01; RAR B.9.2.5.14). Five concentrations were tested with the nominal test concentrations being 0.59, 1.8, 5.4, 17 and 50 mg/kg. Pyriproxyfen was applied to sediment. Control and solvent control (not specified) were included. Each treatment consisted of 12 replicate 600-mL glass vessels filled with 100 ml sediment (= ~4 cm; 162 g wet weight) overlaid with 175 mL clean medium, to which 10 amphipods (8 days old) were added. In addition, for each treatment three more replicates were included for analytical measurements, except for the control that had six additional replicates (of which three for pore water measurements). Medium was laboratory well water. Flow rate was sufficient for 2 volume replacement per day (no continuous flow applied). Feeding was not specified. Survival was determined at day 28 by sieving the sediment and removing the amphipods. Amphipods from four replicates were used to determine the length. Amphipods from the remaining eight replicates were transferred to 300-mL water-only exposure vessels containing a thin layer of silica sand. Reproduction and survival was determined on days 35 and 42 by removing and counting the adults and offspring in each replicate beaker. The length of surviving adults on day 42 was also determined. Water quality measurements (temperature, pH and dissolved oxygen levels) were determined daily levels in one replicate per treatment, and in all replicates on days 0, 28, 29 and 42. On the four days, composite samples per treatment were used to measure total hardness, alkalinity, conductivity and total ammonia concentration in the overlying water. At days 0, 14 and 28, pH and ammonia (as nitrogen) concentration were measured in one pore water sample from each of three additional replicates of the negative control. Analytical measurements were performed on days 0, 14 and 28 on overlaying water and sediment for all treatments, while pore water concentration was only determined in the negative control. Temperature ranged 22-24 °C, pH ranged 6.9-7.5, dissolved oxygen ranged 3.1-7.3 mg/L, ammonia was ≤0.32 mg/L. The mean measured sediment concentrations were: 0.52, 1.5, 5.4, 15 and 44 mg/kg for the 0.59, 1.8, 5.4, 17 and 50 mg/kg, ranging from 85 to 99% of nominal concentrations. The mean measured pore water concentrations were: 0.0012, 0.0039, 0.015, 0.041 and 0.15 mg/L, respectively. The mean measured overlying water concentrations were much lower amounting to 0.00064, 0.0029, 0.0077, 0.017 and 0.091 mg/L on day 0, and decreased to <0.00030, <0.00030, <0.00030, 0.00079 and 0.0035 mg/L on day 28. During the test 85-99% of pyriproxyfen remained associated with sediment/pore water. The biological results are presented in the tables below. The validity criteria were met with control survival exceeding 80% at day 28, length exceeded 3.2 mm at day 28, and >2 young per female were observed in the period 28-42 days There were no significant differences between control and solvent control in growth, survival or reproduction throughout the exposure period (42 days). The only significant effect found was reduced growth after 42 days at the highest treatment with the length at 44 mg/kg being 5.64 mm compared to 6.04 mm for the control organisms. The 42d-NOEC was determined to be 15 mg/kg expressed as mean measured sediment concentration, or 0.041 mg/L when expressed as pore water concentration. The results can be used for classification purposes.

Table B.9.2.5.14-1 Mean percent survival of adult amphipods and mean amphipod growth (length) during the chronic exposure of amphipods (*Hyalella azteca*) to pyriproxyfen on day 28

Mean Measured Sediment Concentration (mg/kg)	Day 28	
	Mean Percent Survival[a] (%)	Mean Length per Amphipod[a] (mm)
Control	99 (3)	5.62 (0.24)
Solvent control	99 (3)	5.54 (0.15)
0.52	98 (5)	5.62 (0.12)
1.5	98 (4)	5.67 (0.23)
5.4	98 (5)	5.40 (0.10)
15	98 (5)	5.35 (0.23)
44	98 (5)	5.32 (0.12)

[a] Standard deviation is presented in parentheses.

Table B.9.2.5.14-2 Mean percent survival of adult amphipods and mean number of offspring released per female amphipod during the chronic exposure of amphipods (*Hyalella azteca*) to pyriproxyfen on day 35

Mean Measured Sediment Concentration (mg/kg)	Day 35	
	Mean Percent Survival[a] (%)	Mean Number of Offspring Released per Female[a]
Control	99 (4)	2.4 (1.9)
Solvent control	98 (7)	5.2 (3.4)
0.52	96 (5)	2.5 (2.0)
1.5	96 (5)	6.4 (6.3)
5.4	99 (4)	6.2 (4.1)
15	96 (5)	4.1 (2.2)
44	96 (5)	5.1 (2.6)

[a] Standard deviation is presented in parentheses.

Table B.9.2.5.14-3 Mean percent survival of adult amphipods and mean number of offspring released per female amphipod during the chronic exposure of amphipods (*Hyalella azteca*) to pyriproxyfen on day 42

Mean Measured Sediment Concentration (mg/kg)	Day 42			
	Mean Percent Survival[a] (%)	Mean Length per Amphipod[a] (mm)	Mean Number of Offspring Released per Female[a]	Mean Male:Female Ratio[a]
Control	96 54)	6.04 (0.38)	5.1 (5.2)	1.1 (0.62)
Solvent control	96 (7)	6.03 (0.25)	8.7 (5.0)	1.1 (0.37)
0.52	95 (5)	6.24 (0.24)	9.8 (6.2)	1.1 (0.73)
1.5	94 (7)	6.08 (0.18)	11 (6.9)	1.6 (1.1)
5.4	99 (4)	6.15 (0.28)	16 (7.0)	1.4 (1.6)
15	96 (5)	5.89 (0.23)	8.8 (2.2)	1.6 (0.53)
44	95 (5)	5.64[b] (0.29)	13 (5.7)	1.5 (0.90)

[a] Standard deviation is presented in parentheses

b Significantly reduced compared to the control, based on Bonferroni's Adjusted t-Test ($p<0.05$)

Table B.9.2.5.14-5 Reduction in mean amphipod growth (length) on day 28 and on day 42

	Day 28 (N=4)		Day 42 (N=8)	
Mean Measured Sediment Concentration (mg/kg)	Mean Length per Amphipod[a] (mm)	% reduction compared to pooled controls	Mean Length per Amphipod[a] (mm)	% reduction compared to pooled controls
Control	5.62 (0.24)	N/A	6.04 (0.38)	N/A
Solvent control	5.54 (0.15)	N/A	6.03 (0.25)	N/A
0.52	5.62 (0.12)	-0.72	6.24 (0.24)	-3.40
1.5	5.67 (0.23)	-1.61	6.08 (0.18)	-0.75
5.4	5.40 (0.10)	3.23	6.15 (0.28)	-1.91
15	5.35 (0.23)[b]	4.13	5.89 (0.23)	2.40
44	5.32 (0.12)	4.66	5.64[c] (0.29)	6.55

[a] Standard deviation is presented in parentheses

[b] N=3, amphipods were not added to this replicate at test initiation and therefore the replicate was omitted from all statistical analysis

[c] Significantly reduced compared to the control, based on Bonferroni's Adjusted t-Test ($p<0.05$)

Hagino and Matsuda (1992a) performed a 21-day non-GLP, non-guideline, semi-static test with the crustacean *Asellus hilgendorfii* to assess effects of pyriproxyfen (purity of 97.2%) on survival and growth (CA 8.2.5.2/01; RAR B.9.2.5.6). Three concentrations were tested with the nominal test concentrations being 0.1, 1 and 10 µg/L. Control was included. Each treatment consisted of single 5-L aquarium filled with 2 L dechlorinated water with 20 crustaceans (juveniles; 1st day of free swimming). Body length was determined weekly. Temperature was 25 °C. Analytical monitoring was not conducted. There were no treatment related effects on survival and behaviour. Mean body length was reduced after 14 days in the 1 and 10 µg/L treatments with respectively 5.6 and 14% compared to the control, and at test end with 5.1%, 0.3% and 5.8% in the 0.1, 1 and 10 µg/L treatments, respectively. The applicant reported a NOEC of 10 µg/L. In the RAR it was concluded that the study is not acceptable, but can be used as supplementary information. The main deficiencies identified

were: the lack of study details, including on feeding, water quality monitoring and statistical analysis, the unreplicated test design and the lack of analytical monitoring. This study is not used for classification purposes.

Hagino and Matsuda (1992d) performed a 19 to 24-day non-GLP, non-guideline, semi-static test with the crustacean *Asellus hilgendorfii* to assess effects of pyriproxyfen (purity of 97.2%) on reproduction (CA 8.2.5.2/02; RAR B.9.2.5.7). Three concentrations were tested with the nominal test concentrations being 0.1, 1 and 10 µg/L. Control was included. Each treatment started with ten 5-L aquaria filled with 2 L dechlorinated water containing one pair of crustaceans. First delivery females (21d-old) were used. After at least 10 days, egg brooding females were transferred to beakers filled with 300 mL test solution. Exposure was continued till juveniles became free living (~ 9 days). Female survival, body length and behaviour, as well as number of hatched juveniles were determined (frequency not reported). Temperature was 25 °C. Analytical monitoring was not conducted. No treatment related effects were observed, and a NOEC of 10 µg/L was reported. In the RAR it was concluded that the study is not acceptable, but can be used as supplementary information. The main deficiencies identified were: the lack of study details, including on feeding and water quality monitoring and the lack of analytical monitoring. The RAR did not report the results. The study is not used for classification purposes.

Marine crustaceans

Machado (1995) performed a GLP-compliant 28-day mysid reproduction study under flow-through conditions with pyriproxyfen (purity of 95.3%; named as Sumilarv) using *Mysidopsis bahia* according to U.S. EPA Pesticide Assessment (FIFRA) Guideline 72-4 (CA 8.2.5.2/06; RAR B.9.2.5.11). The data were reanalysed by Lewis (2016) (CA 8.2.5.2/06b). A range-finding test was conducted. Five concentrations were tested in the definitive test with the nominal test concentrations being 0.63, 1.3, 2.5, 5.0 and 10 µg/L. Control and solvent control (acetone; 100 µL/L) were included. All treatments were conducted in duplicate with each replicate being a 20-L aquarium containing 30 mysids (<24h) divided over two retention chambers. Medium was artificial seawater (total hardness of 20-40 mg/L as $CaCO_3$). Photoperiod of 16h light and 8h dark was applied. Flow rate was sufficient for 11 exchanges per day. Mysids were fed live brine shrimp (*Artemia salina*). On day 17, ten pairs (one male and one female) were each placed in a glass jar with the remaining mysids in each aquarium being placed in one of the initial retention chambers. Males from this pool were used to replace any males that died in the jars but any females dying were not replaced. Mortality, behaviour, and the number of offspring produced by each female were determined daily. At test end, mysids were sacrificed and their body length and dry weights were determined. Water quality (temperature, pH and dissolved oxygen levels) were determined daily in each replicate. Analytical monitoring was performed twice prior to test start and on days 0, 7, 14, 15, 21 and 28 in the dilution water control, the low, middle and high treatments. Temperature ranged 26-27 °C, pH ranged 8.0-8.5, dissolved oxygen ranged 77-114% of saturation, and salinity ranged 24 - 26‰. The mean measured concentrations were: 0.48, 0.81, 1.6, 3.1 and 6.4 µg/L, ranging from 62 to 77% of the nominal values. Measurements on day 0 were excluded, as the measurements (80-113% of nominal) were considered unrepresentative (aquaria did not contain retention chambers, mysids nor food). The results are presented in the table below. There were no significant differences between control and solvent control with respect to the investigated endpoints, i.e. survival, reproductive success, parent length and dry body weight. Treatments were therefore compared to the pooled controls. There were no significant effects on survival, total body length of males or females, and dry body weight of males or females, in the treatments compared to the pooled control. Reproductive success was significantly lower in the 1.6, 3.1 and 6.4 µ/L treatments averaging 0.19, 0.16 and 0.08 offspring/female/reproductive day compared to the pooled control that a reproductive success of 0.32 offspring/female/reproductive day, respectively. The 28d-NOEC was reported as 0.81 µg/L. Lewis (2016) used the data from the study and calculated an EC_{10} of 0.87 µg/L. The RAR reported that the validity criterion, i.e. less than 25% of the females in the control group failed to produce 3 or more young, was met. It was noted that the guideline specifies reproductive success as total number of offspring/female, while in this study the parameter was defined as offspring/female/reproductive day. This was considered acceptable as the number of reproductive days was comparable for all treatments (the sum ranged 105 to 110 days per treatment). The EC_{10} was considered unreliable as the reported confidence interval was very large (95% CL: 0.003 – not

determined). The dossier submitter notes that the limited reporting (only summary values) did not allow to recalculate or evaluate the EC_{10} value for reproduction. Considering above, the study is considered reliable with restrictions, and the 28d-NOEC of 0.81 µg/L can be used for classification purposes.

Table B.9.2.5.11-01 Summary of the survival, reproductive success and growth during the 28-day life-cycle exposure of mysids (*Mysidopsis bahia*) to pyriproxyfen

Mean measured concentration (µg a.s./L)	% survival[1]	Reproductive success[1,2]	Total body length (mm)[1,3]		Dry body weight (mg)[1,3]	
			Males	Females	Males	Females
Control	77	0.31	7.1±0.29	7.0±0.39	0.79±0.12	0.87±0.14
Solvent control	90	0.32	7.1±0.58	6.9±0.40	0.84±0.15	0.85±0.20
Pooled control	83	0.32	7.1±0.49	6.9±0.39	0.81±0.14	0.86±0.17
0.48	83	0.30	7.4±0.43	7.3±0.45	0.80±0.14	0.94±0.18
0.81	92	0.33	7.2±0.40	7.1±0.72	0.76±0.10	0.87±0.23
1.6	75	0.19*	7.2±0.40	6.9±0.33	0.83±0.09	0.88±0.20
3.1	82	0.16*	7.2±0.34	7.0±0.34	0.83±0.13	0.92±0.17
6.4	82	0.08*	7.1±0.42	6.8±0.41	0.78±0.13	0.79±0.15

[1] Mean of 2 replicates

[2] Total number of offspring produced by total number of females per reproductive day

[3] Replicate values = mean ± S.D. (n=15 pairs)

* Significantly different from the pooled control (p≤0.05)

Hagino and Matsuda (1992c) performed a 5-day non-GLP, non-guideline, static test with the marine crustacean *Tigriopus japonicus* to assess effects of pyriproxyfen (purity of 97.2%) on survival and growth (CA 8.2.5.2/03; RAR B.9.2.5.8). Three concentrations were tested with the nominal test concentrations being 0.1, 1 and 10 µg/L. Control and solvent control (DMSO + HCO-40) were included. Each treatment was started by adding 10 females (immediately after brooding) to a petri dish filled with 50 mL test solution. Hatched larvae were distributed over 2 dishes (50 larvae each) containing test solution. Medium was 30‰ artificial seawater. Temperature was 25 °C. Analytical monitoring was not conducted. Observations were made on the number of metamorphoses of nauplii to copepods on day 2 after hatching, the sex ratio on day 5 after hatching, survival rate and size of the test organisms. All nauplii transformed to copepods, and there were no treatment related effects on survival, sex ratio, body length and behaviour. A NOEC of 10 µg/L was reported. In the RAR it was concluded that the study is not acceptable, but can be used as supplementary information. The main deficiencies identified were: the lack of study details, including on water quality, the unreplicated test design at test start, and the lack of analytical monitoring. The RAR did not report the results. The study is not used for classification purposes.

Hagino and Matsuda (1992b) performed a 8-day non-GLP, non-guideline, semi-static test with the marine crustacean *Tigriopus japonicus* to assess effects of pyriproxyfen (purity of 97.2%) on reproduction (CA 8.2.5.2/04; RAR B.9.2.5.9). Three concentrations were tested with the nominal test concentrations being 0.1, 1 and 10 µg/L. Control and solvent control (DMSO + HCO-40) were included. Each treatment was started by adding 10 females to a petri dish filled with test solution. After the first brood, females were transferred individually to a 20-mL vial (= 10 vials per treatment). Temperature was 25 °C. Analytical monitoring was not conducted. Observations were made on female survival and behaviour, as well as the number of larvae hatching between the first to the third brood. No treatment related effects on behaviour and survival of females, and number of hatched larvae were reported. A NOEC of 10 µg/L was reported. In the RAR it was concluded that the study is not acceptable, but can be used as supplementary information. The main deficiencies identified

were: the lack of study details, including on water quality, the unreplicated test design at test start, and the lack of analytical monitoring. The RAR did not report the results. The study is not used for classification purposes.

Freshwater insects

Biester (2011) perfomed a 30-day GLP-compliant, non-guideline, semi-static test with the mayfly *Cloeon dipterum* to assess effects of [phenoxyphenyl-^{14}C]-pyriproxyfen (radiochemical purity: 100%) on survival, growth and emergence (CA 8.2.5.2/07; RAR B.9.2.5.12). Four concentrations were tested with the nominal test concentrations being 0.01, 0.03, 0.10 and 0.30 µg/L. Control and solvent control (acetonitrile) were included. All treatments were conducted in quintuplicate with each replicate being a 2-L glass beakers containing 1 L medium, pieces of stainless steel mesh that served as substrate, and 8 mayfly nymphs. The mayfly nymphs were collected from wild populations of microcosms and were acclimated at least 6 days at 15-18 °C prior to test initiation. Medium was natural, filtered water originated from Lake Constance (total hardness: 152-160 mg/L as $CaCO_3$; pH: 7.78-8.20). Nymphs had a mean length: 0.69 ± 0.19 mg and mean dry weight 5.29 ± 0.42 mm at test start (based on sample of 20 nymphs). Test organisms were fed daily a mixture of fish flake suspension and the unicellular green alga *Ankistrodesmus falcatus*. Nymphs were transferred to vessels with fresh medium on days 2, 5, 7, 9, 12, 14, 16, 19, 21, 23, 26 and 28. Mortality, behaviour, moulting and emergence were determined daily. Water quality (temperature, pH and dissolved oxygen levels) were determined at test start, and in fresh and aged test solutions during renewal. Hardness was determined at test start in fresh solutions and at test end in the aged solutions of the control and highest treatment. Analytical monitoring (LSC) was performed at days 0, 7, 16 and 26 in fresh solution, and on days 2, 19, 19 and 28 in aged solutions. Parent concentration was determined by HPLC in the highest treatment. Temperature ranged 16-18 °C, pH ranged 8.3-8.6, dissolved oxygen ranged 7.9-9.7 (>60% of saturation), hardness ranged 156-160 mg/L as $CaCO_3$. The geometric mean measured concentrations were: 0.008, 0.024, 0.079 and 0.234 µg/L based on total radioactivity were. There were no large differences between fresh (78-98% of nominal) and aged test solutions (65-95%). The mayfly nymphs hatched between 5 and 30 days after their addition and the mean emergence in the controls was greater than 70%, meeting 'validity criteria'. Survival, emergence and development rate of control and solvent control did not differ significantly. Treatments were therefore compared to the pooled controls. Mean survival rates amounted to 0.95, 0.95, 1.00, 1.00, 1.00 and 0.95 in the control, solvent control and the 0.008, 0.024, 0.079 and 0.234 µg/L treatment, respectively. The mean emergence rates amounted to 0.95, 0.95, 1.00, 1.00, 1.00 and 0.93, respectively. The mean development rates amounted to 0.0644, 0.0648, 0.0671, 0.0678, 0.0633 and 0.0688 day^{-1}, respectively. There were no significant effects on survival, emergence and development rate compared to the pooled control. The 30d-NOEC was reported as 0.234 µg/L. The RAR noted that the study design was acceptable generally following OECD TG 219, i.e. sediment-water chironomid toxicity test with spiked. There were, however, two major deficiencies identified: (1) the analytical method was based on total radioactivity. Consequently, as in the aged solution of the highest treatment, pyriproxyfen represented 62-78% of radioactivity, the geometric mean measured test concentration reported by the applicant was an overestimate. The correct mean measured test concentration was 0.196 µg/L, yielding a 30d-NOEC of ≥0.196 µg/L; (2) the developmental stage of the nymphs was unknown. The applicant attempted to infer developmental stage from literature sources, which was unsuccessful as the parameter 'wing pad development' was not measured, and the parameters 'larval length' and 'larval weight' were rather variable (potentially mix of developmental stages and sexes). Based on total emergence within 30 days, it was considered in the RAR that later larval stages were used. This is relevant as individuals should be exposed through the entire larval stage (i.e. OECD TG 219 requires the use of first instar larvae). Considering all above, the study was considered unreliable. The 30d-NOEC of ≥ 0196 µg/L is not used for classification purposes.

Putt (2003) performed a GLP-compliant 28-day sediment-water chironomid toxicity test under static conditions with [pyridyl-2,6-^{14}C]-pyriproxyfen (radiochemical purity: 99.6%) and pyriproxyfen (purity: 97.9%) using the non-biting midge *Chironomus riparius* according to OECD TG 219 (CA 8.2.5.3/01; RAR B.9.2.5.13). Five concentrations were tested with the nominal test concentrations being 2.5, 5.0, 10, 20 and 40 µg/L (based on range-finding test). Control and solvent control (acetone; 100 µL/L) were included. Each

treatment consisted of eight replicate 600-mL glass beaker filled with 149 g (= 1.5 cm) natural sandy sediment (2.4% organic carbon; pH of 6.2) and overlaid with 300 mL clean medium (well water; hardness of 49-50 mg/L as $CaCO_3$; pH of 7.4-7.7). Test vessels were allowed to reach equilibrium for 7 days, after which 20 larvae (~2 days old) were added to each test vessel. 24 hours after addition of the larvae, the test substance was spiked to the overlaying water, i.e. radiolabelled substance to the lowest two treatments, and a mixture of radiolabelled and non-labelled for the three highest treatments. Gentle aeration was applied throughout the test except for the initial 24h to allow larval settlement. Midges were daily fed with ground fish flakes. Emergence was observed daily, and emerged midges were immediately removed. Following test end (day 28), vessels were kept to distinguish between delayed emergence and mortality, until there were no emergences for 7 consecutive days. Water quality (temperature, pH and dissolved oxygen levels) were determined at test start, on days -1, 0, 28, and termination of test vessels (up to day 37), and also daily measured in a replicate vessel. On days 0, 7, 14 and 28 one of the four analytical replicates was sacrificed per sampling time to determine: (i) total radioactivity in sediment and overlaying water of all treatments using LSC; (ii) total radioactivity in pore water of the 2.5, 10 and 40 µg/L treatments using LSC; (iii) parent concentrations in overlaying water and sediment of the 2.5, 10 and 40 µg/L treatments using HPLC/RAM. Temperature ranged 19-22 °C, pH ranged 7.6-8.2, dissolved oxygen ranged 7.2-9.3 (>60% of saturation). Total radioactivity in overlying water amounted at day 0 (1 h post application) to 2.2, 4.5, 9.6, 19 and 35 µg/L for the 2.5, 5.0, 10, 20 and 40 µg/L treatments, respectively, corresponding to 88-96% of nominal test concentrations. The total radioactivity in overlaying water decreased to 17-27% of nominal by day 7 and remained in that order throughout the remainder of the study. HPLC/RAM analysis of overlying water showed that at day 0 almost all extracted radioactivity (≥98% of applied radioactivity (% AR)) was associated with pyriproxyfen, while at days 7, 14, and 28 the metabolite PYPAC amounted to, respectively, 68%, 81% and 100% AR. In sediment, total radioactivity amounted at day 0 to <LOQ, 1.6, 2.7, 5.0 and 10 µg/kg for the 2.5, 5.0, 10, 20 and 40 µg/L treatments, respectively, and increased by day 7 to 1.2, 3.0, 9.0, 18 and 29 µg/kg (19 to 36% AR). Sediment concentrations decreased slightly and ranged 9-13% AR by day 28. HPLC/RAM analysis of sediment showed that at days 7, 14 and 28 pyriproxyfen represented ≤41% AR, with the remainder was associated with the metabolites PYPAC and '4- OH-Pyr. In pore water, total radioactivity amounted at day 0 to 0.057, 0.38 and 0.77 µg/L in the 2.5, 10 and 40 µg/L treatments, respectively, and increased at day 28 to 0.66, 2.7 and 9.4 µg/L. There was no parent analysis in pore water. The applicants did not report mean measured test concentrations. The biological results are presented in the table below. Survival, emergence and developmental rate in the control and solvent control did not differ significantly. Treatments were therefore compare to the pooled control. In the two highest treatments, i.e. 20 and 40 µg/L, significant reduction in the mean development rate of pooled sexes was observed compared to the pooled control. Inclusion of the 2 females that emerged post day 28 (one per treatment) did not affect the outcome. The 28-d NOEC was reported as 10 µg/L (nominal). The RAR noted some minor deviations in study design that did not affect the study outcome. The NOEC based on nominal test concentrations was initially accepted in the RAR as the initiation actual pyriproxyfen concentrations in overlaying water corresponded to 86-95% of the nominal test concentrations in the 2.5, 10 and 40 µg/L treatments, respectively. However, pyriproxyfen concentrations in overlaying water dropped rapidly (due to degradation/dissipation) and, as noted in the RAR, the nominal test concentrations do not represent the real exposure. In the RAR a geometric mean measured NOEC of 0.031 mg/L was derived, and an EC_{10} of 23 µg/L (nominal) based on development rate was determined. As confidence intervals could not be determined, the EC_{10} was considered unreliable. The dossier submitter notes that the unit of the NOEC reported in the RAR is erroneous and should be µg/L instead of mg/L, resulting in a 28d-NOEC of 0.031 µg/L based on mean measured concentrations in overlaying water. The dossier submitter further notes that exposure of chironomid larvae occurs predominantly via pore water, and expressing the NOEC based on pore water is considered a better representation of actual exposure. Total radioactivity measurements of pore water show an opposite pattern compared to overlaying water, with total radioactivity levels being very low at test start and increasing towards test end. To what extent the radioactivity at test end corresponds to pyriproxyfen is unknown, but considering sediment and overlaying water data, it is reasonable to assume that, at best, less than 50% represent pyriproxyfen. Pore water concentrations would thus be considerably lower than overlaying water concentrations, but an exact value cannot be derived. The results are considered reliable with restrictions, and can be used for classification purposes.

Table B.9.2.5.13-1 Summary of results (mean percent emergence and mean development rates) calculated at test termination (day 28) of the midge (*Chironomus riparius*) full life-cycle exposure with pyriproxyfen

Nominal concentration (µg a.s./L)	Mean percent emerged (%)	Mean development rates		
		Male midges	Female midges	Male/female midges
Control	86	0.0567	0.0505	0.0536
Solvent control	84	0.0587	0.0554	0.0571
Pooled control	85	0.0577	0.0530	0.0553
2.5	80	0.0636	0.0553	0.0594
5.0	78	0.0613	0.0540	0.0572
10	88	0.0606	0.0519	0.0551
20	74 (75)[1]	0.0522 (0.0522)[1]	0.0486 (0.0484)[1]	0.0496* (0.0487)[1]
40	76 (78)[1]	0.0455 (0.0455)[1]	0.0439 (0.0436)[1]	0.0446* (0.0438)[1]

[1] Figures in brackets are results of extended exposure (day 37)

* Statistically significantly different compared to the pooled control data ($p<0.05$).

Roessink (2018) performed an indoor microcosm experiment (that is not discussed here), supplemented with three 28-day single species semi-static toxicity test (CA 8.2.5.2/08; RAR B.9.2.5.15) . Test species were aquatic insects, i.e. the mayfly *Cloeon dipterum*, the water bugs *Plea minutissima* and the damselflies *Coenagrion pulchellum* and *Enallagma cyanthigerum* (tested as mixture of two species), that were collected 5 days prior to testing from uncontaminated experimental ecosystems. As there are no standardized test guideline for the above species, experiments were based on available OECD TG for aquatic invertebrates (OECD TG 219, 223 and 211). Test substance was pyriproxyfen technical grade (purity 99.2 – 100%). In all three test, the nominal test concentrations were 0.1, 0.4, 1.6, 6.25, 25 and 100 µg/L. Controls and solvent controls (acetone) were included. Medium was aerated groundwater. Mayflies and water bugs were tested in 1.5-L glass beakers covered with a glass lid to which ~1L medium was added, and 10 individuals per test vessel. Controls and solvent controls were performed in quintuplicate and the treatments in triplicate in the mayfly and water bug tests. The cannibalistic damselflies were tested individually in 100-mL glass tubes filled with ~100 mL medium. Control and solvent control consisted of 30 damselflies, and the treatments of 15 damselflies. Surplus damselflies (15 individuals) were used as a representative sample for length and weight measurements and identification to species level. All test vessels were placed in an acclimatised room. Test medium was renewed twice weekly. Water quality (temperature, dissolved oxygen and pH) was determined in the control and highest treatments before and after every renewal. Analytical measurements (HPLC-MS/MS) were conducted in in the dosing solutions and in each test at days 0, 4, 7, 11, 14, 18, 21, 25 and 28. Immobility and mortality of the test organisms were recorded at days 7, 14, 21 and 28, the first defined as limited movement after tactile stimulation, and the latter as no response of any kind after tactile stimulation for a time period of 15 seconds. Emergence and abnormal effects were examined on all days when the test systems were handled for refreshment of the medium. Emerged and dead animals were removed during examination. The water quality parameters in the mayfly, water bug and damselfly tests were as follows: temperature ranged 6.8-19.8, 16.8-20.1 and 19.6-20.9°C, respectively; pH ranged 6.9-8.6, 7.1-8.9 and 7.7-9.6, respectively; dissolved oxygen ranged 88-103, 70-130 and 99-140% of saturation, respectively. The mean measured (time weighted average)

concentrations were: 0.034, 0.11, 0.46, 3.08, 16.70 and 72.58 µg/L in the mayfly test; 0.034, 0.12, 0.50, 3.34, 17.29 and 86.35 µg/L in the water bug test; and 0.035, 0.10, 0.29, 1.68, 11.69 and 66.16 µg/L in the damselfly test, respectively. The results are discussed per species. In the mayfly test, control and solvent control did not significantly differ with respect to mortality (20 vs. 10%); Average length and average dry weight determined in the representative sample were 3.48±0.6 mm and 0.08±0.04 mg, respectively. At day 14, mortality amounted to ~50% and 100% in the 16.70 and 72.58 µg/L treatments, respectively. Emergence occurred only at day 28 in the control (1 mayfly) and solvent control (2 mayflies). No sub-lethal effects were observed. For mayflies, a 28d-LC_{10} of 14.71 µg/L (mm) and a 28d-NOEC of 16.7 µg/L (mm) were reported. In the water bug test, control and solvent control did not significantly differ with respect to mortality (6 vs. 8%); Average length and average dry weight determined in the representative sample were 2.36±0.12 mm and 0.84±0.10 mg, respectively. At day 28, mortality in the highest treatment was 6.7%. No sub-lethal effects were observed For water bugs, an LC_{10} could not be derived, the 28d-NOEC was reported as ≥86.35 µg/L (mm). In the damselfly test, control and solvent control did not significantly differ with respect to mortality (3.3 vs. 0%); Average length and average dry weight determined in the representative sample were 10.8±1.2 mm and 2.52±0.82 mg, respectively. There was no dose response relationship with regard to mortality. From day 21 onward, sub-lethal effects were observed with individuals in the 0.29, 1.68, 11.69 and 66.16 µg/L treatments showing dark coloration. The darker individuals were reported to react similarly to tactile stimuli as animals with a lighter coloration. There were no treatment related effects reported on body length, dry weight biomass or growth rates. Emergence did not occur. For damselflies, a 28d-EC_{10} of 0.21 µg/L (mm) and a 28d-NOEC of 0.6 µg/L (mm) were reported based on the darker coloration. In the RAR, it was noted that this study corresponded to a prolonged acute study as the maximum response appeared to be reached after 14 days for mayflies and possible sub-lethal effects on development (e.g., wing pad length) were not monitored. It was further noted that the dry weight of mayflies and damselflies in the representative samples highly varied (samples were not sexed), and that the damselflies were a mix of two different species that tend to differ in emerge period. The NOEC of 16.7 µg/L (mm) obtained for mayflies was considered as reliable and supporting that aquatic insects are less sensitive than *Daphnia magna*. The dossier submitter considers the data for damselflies as unreliable, as two species were tested that might differ in sensitivity. The data reported for mayflies and water bugs can be used for classification purposes.

Concluding on the parent

From the above summaries it can be concluded that there are eleven studies available for aquatic invertebrates of which five were assessed as acceptable for classification purposes. These studies yielded six reliable effect concentrations (mean measured) for six aquatic invertebrate species that can be grouped into: freshwater crustaceans with a 21d-EC_{10} of 0.0000088 mg/L for the water flea *Daphnia magna* and a 42d-NOEC of 0.041 mg/L for the amphipod *Hyalella Aztec*; marine crustaceans with a 28d-EC_{10} of 0.00087 mg/L for the mysid *Mysidopsis bahia*; and freshwater insects with a 28d-NOEC of 0.000031 mg/L for the midge *Chironomus riparius*, a 28d-EC_{10} of 0.0147 mg/L for the mayfly *Cloeon dipterum*, and a 28d-NOEC of ≥0.0864 mg/L for the water bug *Plea minutissima*. *Daphnia magna* is clearly the most sensitive species of the tested aquatic invertebrate species, and the 21d-EC_{10} of 0.0000088 mg/L is considered key data for classification purposes.

Selected aquatic invertebrate studies with degradation products

Rhodes and Bucksath (1996) performed a GLP-compliant 21-day daphnia reproduction study under flow-through conditions with 4'-OH-Pyr (purity of 99.1%) and PYPAC (purity of 100%) using *Daphnia magna* according to U.S. EPA Pesticide Assessment Guidelines, 72-4(b) (CA 8.2.5.1/02; RAR B.9.2.5.2). Both substances were tested separately. Limit test with a nominal test concentration of 5 µg/L. Control and solvent control (0.10 mL/L acetone) were included. All treatments were conducted in quadruplicate. Each replicate consisted of 10 daphnid (<24h) incubated in a 1-L test vessel with 800 mL medium. Medium was hard blended water. Feeding was twice daily (mixture of algae, yeast and fish food). Parent mortality, abnormal effects and first-brood were observed daily. Reproduction was observed three times a week. Parent length and dry weight were determined at test end. Water quality (temperature, dissolved oxygen and pH) was determined at days 0, 4, 7, 14 and 21 in alternating duplicate replicates. Analytical measurements (HPLC) were conducted on the

same days. Temperature ranged 19-20 °C, pH ranged 6.5-8.6, dissolved oxygen ranged 75-99% of saturation. The mean measured concentrations were 4.74 and 4.39 µg/L for PYPAC and 4'-OH-Pyr, respectively, corresponding to 88 and 95% of the nominal test concentrations. The results are presented in the table below.

Table B.9.2.5.2-01 Biological effects for control, solvent control, pooled control, PYPAC and 4'-OH-Pyr

Treatment	Mean measured concentration [µg/L]	Adult survival		Reproduction/replicate [b]		Mean adult length [mm]	Mean adult weight [mg]
		#	[%]	# total young	Time to 1st brood [d]		
Control	<1.26	40	100	1325	8	4.38	0.83
Solvent control	<1.26	40	100	1170	8	4.28	0.83
Pooled control	-	-	100	-	-	4.33	0.83
PYPAC	4.74	39 [a]	100	1240	8	4.32	0.82
4'-OH-Pyr	4.39	37 [a]	95	926	9	4.28	0.83

a Number of daphnids assessed: 39 due to mortality caused by mechanical/physical error during changeover procedure and not attributable to exposure

b each replicate contained 9-10 daphnids at test initiation

There were no significant differences between control and solvent control, except for the parameter "young per adult reproductive day". Statistical analysis was therefore performed using the solvent control for the parameter "young per adult reproductive day" only, while for all other parameters comparison was made with the pooled controls. No significant effects were detected for daphnids exposed to PYPAC, yielding for PYPAC a 21d-NOEC of ≥4.74 µg/L. Daphnids exposed to 4'-OH-Pyr had significantly less "young per adult reproductive day" and a delayed "days to first brood", yielding for 4'-OH-Pyr a 21d-NOEC of <4.39 µg/L. In the RAR, it was noted that the validity criteria (adult mortality <20%, mean number of living offspring per surviving parent ≥60) were met, and the study was considered acceptable. The results can be used for classification purposes.

Shaw (2015a) performed a GLP-compliant 21-day daphnia reproduction study under flow-through conditions with 4'-OH-Pyr (purity of 99.2%) using *Daphnia magna* according to OECD TG 211 (CA 8.2.5.1/03; RAR B.9.2.5.2). The nominal test concentrations were 0.22, 0.44, 0.88, 1.8 and 3.5 µg/L. Control and solvent control (0.10 mL/L acetone) were included. All treatments were conducted in quadruplicate. Each replicate consisted of 10 daphnid (<24h) incubated in a 1.6-L test vessel. Flow rate was sufficient for 90% solution exchange per 9 hours. Medium was fortified well water. Feeding was three times daily (mixture of algae, yeast and fish food). Parent mortality, abnormal effects and first-brood were observed daily. Reproduction was observed minimal three times a week. Parent length was determined at test end. Water quality (temperature, dissolved oxygen and pH) was determined in each test vessel at test start and weekly thereafter. And in alternating test vessels of each exposure daily. Analytical measurements (HPLC-MS/MS) were conducted on the days 0, 7, 14 and 21 in two replicates per treatment. Temperature ranged 20-21 °C, pH ranged 7.6-8.6, dissolved oxygen ranged 6.0-8.6 (67-96% of saturation). The mean measured concentrations were 0.13, 0.39, 0.63, 1.4 and 2.9 µg/L ranging from 13 to 16% of the nominal test concentrations. The results are presented in the table below.

Table B.9.2.5.3-01 Biological results in the 21-day toxicity test of 4'-OH-Pyr to *Daphnia magna*

Treatment group	Nominal concentration [µg/L]	Mean measured concentration [µg/L]	Mean % survival [%]	Reproduction		Mean total adult body length [mm]
				Mean # offspring/ female (± SD)	% Reduction [a]	
Control	0.00	<0.02	95	151 (± 16)	-	4.94
Solvent control	0.00	<0.02	95	148 (± 5)	-	4.87
Pooled control	-	-	95	149 (± 11)	-	4.91
4'-OH-Pyr	0.22	0.13	90	153 (± 14)	-2.7	4.88
	0.44	0.39	93	168 (± 17)	-13	4.95
	0.88	0.36	85	171 (± 23)	-15	5.01
	1.8	1.4	93	151 (± 16)	-1.3	4.89
	3.5	2.9	95	139 (± 13)	6.7	4.80

a compared to pooled control, as solvent control was not statistically significantly different from untreated control; calculated by RMS

There were no significant differences between control and solvent control, and comparison was made to the pooled control. The largest effect was observed for the highest treatment showing a reduction of 6.7% in the mean number of offspring/ surviving female compared to the pooled control. This effect was not statistically significant. Overall no significant effects were observed for any of the endpoints. The 21-d NOEC for 4'-OH-Pyr for *Daphnia magna* was determined to be 2.9 µg/L (mean measured). In the RAR, it was noted that the validity criteria (adult mortality <20%, mean number of living offspring per surviving parent ≥60) were met, and the study was considered acceptable. The results can be used for classification purposes.

Discussion on degradation products

In the RAR additional aquatic toxicity tests are available for pyriproxyfen degradation products (PYPAC and 4'-OH-Pyr, but also PYPA and DPH-Pyr) in the water flea *Daphnia magna* but also other aquatic species such as fish. As the aim is to demonstrate that the formed degradation products are classifiable as hazardous to the aquatic environment, only critical studies are shown above. As for the parent substance, *Daphnia magna* appears to be a very sensitive species for the degradation products with the lowest chronic effect concentrations being a 21d-NOEC of 4.74 µg/L for PYPAC and a 21d-NOEC of 2.9 µg/L for 4'-OH-Pyr.

11.6.3 Chronic toxicity to algae or other aquatic plants

Please see section 11.5.3 where the studies have been discussed in detail.

11.6.4 Chronic toxicity to other aquatic organisms

In order to assess the potential endocrine effects of pyriproxyfen, anonymous (2012) performed a GLP-compliant amphibian metamorphosis assay with pyriproxyfen (purity 99.5%) using the African clawed frog (*Xenopus laevis*) following US OPPTS 890.1100 and OECD TG 231 guidelines (CA 8.1.4/01; RAR B.9.1.5). Three concentrations were tested with the nominal test concentrations being 3.0, 30 and 300 µg/L. Control and solvent control (not specified) were included. Medium was FETAX solution (total hardness of 180-200 mg/L as $CaCO_3$). All treatments, including controls, were tested in quadruplicate and contained per 2.5-gallon exposure aquarium containing 20 tadpoles (stage 51; pre-exposure day 13) in 6.5 L medium (total of 80 tadpoles/treatment). Flow rate was sufficient for ~6.1 aquarium volumes exchange per day. During the pre-exposure and in-life exposure periods, tadpoles were fed Xenopus Express Tadpole Food with the daily feeding rates being increased as the study progressed to account for growth. Water quality (temperature, dissolved

oxygen and pH) was determined in all test vessels at day 0, and in alternating replicates daily thereafter. Total hardness, total alkalinity and specific conductance were measured in one replicate of the control, low and high test concentrations on day 0 and in alternating replicates weekly thereafter. Temperature was continuously monitored in one of the solvent controls. Analytical monitoring of the solutions (not specified) was performed at days 0 in all test vessels, and at days 8, 9, 10, 15 and 21 from alternating replicates from the treatments and controls. Survival and behavioural assessment was performed daily for all test vessels. On day 7, five tadpoles were removed randomly from each test vessel and euthanized to determine the developmental stage, determine body weight, snout-vent length and hind limb length. At test termination (day 21), the remaining tadpoles were removed from the test vessels and euthanized to determine the developmental stage, determine body weight, snout-vent length, hind limb length and histological analyses (thyroid gland) of five tadpoles per replicate. Temperature ranged 22-24°C, pH ranged 7.2-8.2, dissolved oxygen ranged 3.6-8.8 mg/L (42 and 100% saturation, respectively), total hardness ranged 140-160 mg/L (as $CaCO_3$), the alkalinity ranged 74-80 mg/L (as $CaCO_3$), and specific conductance ranged 1600-1800 µmhos/cm. Mean measured concentrations ranged from 57 to 75% of the nominal levels and defined the treatment levels tested as 1.7, 23 and 210 µg/L. No mortality was reported in the control, solvent control, or any of the treatments. In the highest treatment tadpoles showed reduced food consumption throughout the exposure, and their size was smaller compared to the control from day 6 on. In the 1.7 µg/L treatment, two tadpoles were deformed (spinal curvature) from day 13 on. Control and solvent control did not differ significantly for any of the endpoints, and comparison was made to the pooled control. In the table below the results obtained for the apical endpoints are shown.

Table CA 8.1.4/01-1 Results for apical endpoints

	Developmental stage [a)]	Snout-vent length (mm) [b)]	Hindlimb length (mm) [b)]	Normalized hindlimb length [b)]	Whole body wet weight (g) [b)]
Day 7					
Control	54	20.32	2.96	0.14	0.5442
Solvent control	54	19.37	2.84	0.15	0.5014
Pooled control	54	19.84	2.95	0.15	0.5228
1.7 µg/L treatment	54	20.74	2.75	0.13*	0.5945
23 µg/L treatment	54	19.47	2.62*	0.13*	0.5070
210 µg/L treatment	52*	13.49*	1.48*	0.11*	0.1698*
7d-NOEC	**23 µg/L**	**23 µg/L**	**1.7 µg/L**	**<1.7 µg/L**	**23 µg/L**
Day 21					
Control	60	26.59	21.36	0.84	1.5078
Solvent control	60	27.23	22.20	0.85	1.5921
Pooled control	60	26.91	21.78	0.84	1.5499
1.7 µg/L treatment	60	27.42	21.74	0.82	1.6330
23 µg/L treatment	60	26.16	20.33*	0.80	1.4509
210 µg/L treatment	55*	18.96*	3.700*	0.19*	0.4271*
21d-NOEC	**23 µg/L**	**23 µg/L**	**1.7 µg/L**	**23 µg/L**	**23 µg/L**

* Significantly different from pooled control at 5% level
a) Mean of medians per replicate
b) Mean of means per replicate

Effects on thyroid gland histopathology were not observed at any treatment level. Mild follicular cell hypertrophy or mild follicular cell hyperplasia was diagnosed in 15 – 30% of the animals from the control, the solvent control and at 1.7 and 23 µg/L. No animals in the 210 µg/L exhibited follicular cell hypertrophy and only two animals exhibited follicular cell hyperplasia (one from the 210 µg/L group and one from the stage-matched controls). Given the consistency in histologic appearance between the 210 µg/L treatment group and stage-matched controls, the discrepancy in the incidence of follicular cell hyperplasia was attributed to

differences in growth patterns at different developmental stages. Based on the lack of advanced development, asynchronous development or changes in thyroid histology, it is concluded that pyriproxyfen was thyroid inactive.

Table CA 8.1.4/01-02 Histological findings in thyroid (number of animals in which the finding was recorded)

Finding	Control	Solvent control	1.7 µg/L treatment	23 µg/L treatment	210 µg/L treatment	Stage-matched control
Number of animals assessed (n)	20	20	20	20	20	5
Thyroid gland hypertrophy	1	2	1	0	0	1
Thyroid gland atrophy	0	1	0	0	0	0
Follicular cell hypertrophy	5	4	5	6	0	0
Follicular cell hyperplasia	5	4	6	3	1	1
Increased follicular lumen area	2	1	1	2	1	0
Decreased follicular lumen area	0	0	0	0	0	1
Changes in colloid quality	1	0	0	0	0	0
Lymphocytic inflammation	1	0	0	0	0	0
Pharyngeal epithelial necrosis	7	10	10	10	0	0

The lowest 28d-NOEC was 0.0017 mg/L based on hindlimb length. The study did not allow to calculate an EC_{10}. In the RAR, it was noted the study met the validity criteria, and that while one out of the ten performance criteria was not met and two could not be verified, this was not considered to invalidate the test. The performance criteria that was not met concerned the maintaining of the test concentrations at ≤20% CV over the test period. For the lower two treatments CV was 30 and 26%, respectively, but as the concentration gradient was maintained this was not considered to have affected the outcome of the stud. The inter-replicate/inter-treatment differentials in pH (≤0.5) and temperature (0.5°C) could not be verified were as only ranges for the entire test period were reported. The study was considered valid and reliable in the RAR, and the results can be used for classification purposes.

Concluding

From the above summary it can be concluded that there is one reliable study available for the African clawed frog (*Xenopus laevis*) reporting a 21d-NOEC of 0.0017 mg/L (mean measured). This value will be used for classification purposes.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Reliable acute studies with pyriproxyfen are available for three trophic levels: algae/aquatic plants, aquatic invertebrates and fish. The lowest effect concentration per group is: a 72h-E_rC_{50} of 0.111 mg/L for the green algae *Raphidocelis subcapitata*; a 96h-EC_{50} of 0.065 mg/L for the mysid *Mysidopsis bahia*; and a 96h-LC_{50} of

>0.270 mg/L for the bluegill sunfish *Lepomis macrochirus*. All endpoints are expressed as mean measured test concentrations.

From these data it is clear that aquatic invertebrates are the most acutely sensitive trophic group. Based on the lowest EC_{50} of 0.065 mg/L that is below 1 mg/L, pyriproxyfen should, in accordance with tables 4.1.0(a) and 4.1.3 (according to CLP guidance V5.0 July 2017, p. 505-509) be classified as Aquatic Acute 1; H400, with an M-factor of 10.

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

With regard to the rapid degradability of pyriproxyfen, several studies are available for the aquatic environment. Photodegradation of pyriproxyfen in water was observed under laboratory condition, but this is likely to be limited under realistic environmental conditions. Pyriproxyfen was shown to be hydrolytically stable under environmental conditions and not readily biodegradable with degradation amounting to 0.691% (based on oxygen consumption) after 28 days in a modified MITI I test. In a surface water simulation degradation study with radiolabelled pyriproxyfen, ultimate degradation was limited with CO_2 production at test end amounting to 12.4 to 32.4% of applied radioactivity. Primary degradation occurred, with the DT50 for pyriproxyfen amounting in the low and high dosing system to 10.6 and 30.8 days, respectively, when normalized to the European standard temperature of 12 °C (non-normalized values were 5.0 and 14.5 days at the laboratory test temperature of 20 °C). Thus, in one of the treatments the half-life limit of <16 days is exceeded. Furthermore, 24 degradation products were detected, of which six (>10% AR) were identified as 4'-OH-Pyr, DPH-Pyr, PYPAC, PYPA, POP and 4'-OH-POP. At least two of these degradation products appear to be classifiable based on, for example, the very low chronic effect concentrations reported for the aquatic invertebrate species *Daphnia magna*, i.e. a 21d-NOEC of 4.74 µg/L for PYPAC (Rhodes et al., 1996; CA 8.2.5.1/02; B.9.2.5.2) and a 21d-NOEC of 2.9 µg/L for 4'-OH-Pyr (Shaw, 2015a; CA 8.2.5.1/03; B.9.2.5.3). Furthermore the other 18 degradation products remained unidentified and could potentially be classifiable as hazardous to the aquatic environment. Considering all above, pyriproxyfen is considered as **not rapidly degradable** for classification purposes.

With regard to the bioaccumulation potential of pyriproxyfen, the log K_{ow} was reported to be 4.85, which exceeds the exceed the threshold of log K_{ow} ≥4. Experimentally determined BCF values are available that contain some uncertainties. The highest BCF values were obtained for the bluegill sunfish *Lepomis macrochirus* using radiolabelled pyriproxyfen. The whole fish BCF values amounted to 1379 and 1495 L/kg when based on steady-state calculations and 1489 and 1653 L/kg when based on a simultaneous kinetic fit of uptake and depuration. As the analytics were based on total radioactivity, it remains uncertain if (all) radioactivity can be attributed to pyriproxyfen, potentially leading to an overestimation of pyriproxyfen bioaccumulation. Fish tissue analysis in the respective study showed that on days 21 and 28 of the uptake phase pyriproxyfen plus 4'-OH-pyriproxyfen and its conjugates together accounted for 37-70% of the radioactive residue, suggesting these BCF values overestimate to some extent the bioaccumulation of pyriproxyfen. However, the BCF values were not normalized to 5% lipid content, and were not corrected for growth. These factors could lead to an underestimation. In the second bioaccumulation study, steady-state lipid-normalized BCF values of 669 and 512 L/kg were reported for the carp *Cyprinus caprio* based on parent substance analysis. There are uncertainties associated with this study as sampling during the uptake phase was reduced and the duration of the depuration phase was shorter than recommended. Also the reporting was limited not allowing to calculate BCF values based on kinetic fitting. Altogether, both studies show that the BCF values in any case exceed the threshold of BCF ≥500 L/kg_{ww}. Considering the log K_{ow} and BCF values it can be concluded that pyriproxyfen has a **potential for bioaccumulation**.

Reliable chronic studies with pyriproxyfen are available for the following trophic levels: algae/aquatic plants, aquatic invertebrates, frogs and fish. The lowest effect concentration per group is: a 72h-NOEC of 0.020 mg/L for the green algae *Raphidocelis subcapitata*; a 21d-EC_{10} of 0.0000088 mg/L for the water flea *Daphnia magna*; a 21d-NOEC of 0.0017 mg/L for the African clawed frog *Xenopus laevis*; and a 189d-NOEC of 0.0027 mg/L for the medaka *Oryzias latipes*. All endpoints are expressed as mean measured test concentrations.

From these data it is clear that aquatic invertebrates are the most chronically sensitive trophic group. It is noted that for the acutely most sensitive species, i.e. the mysid *Mysidopsis bahia* (96h-EC_{50} of 0.065 mg/L for mysids

versus 48h-EC_{50} of 0.40 mg/L for water fleas), chronic data are available. The chronic data show that mysids (28d-EC_{10} of 0.00087 mg/L) are less sensitive than water fleas (21d-EC_{10} of 0.0000088 mg/L).

Considering that pyriproxyfen is not rapidly degradable and has a potential for bioaccumulation, and based on the lowest EC_{10} of 0.0000088 mg/L that is below 0.1 mg/L, pyriproxyfen should, in accordance with tables 4.1.0(b) and 4.1.3 (according to CLP guidance V5.0 July 2017, p. 505-509) be classified as Aquatic Chronic 1; H400, with an M-factor of 10000.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Aquatic Acute category 1; H400 Very toxic to aquatic life

Acute M-factor = 10

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

Chronic M-factor = 10000

Pictogram code: GHS09

Label hazard statement: H410 Very toxic to aquatic life with long lasting effects

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Table 74: Summary table of data concerning hazardous properties of the substance for the ozone layer

Type of study/data	Test substance,	Relevant information about the study	Observations	Reference
Stability in air	NA	Calculation only, using the Atkinson equation (AOPWIN v. 1.92 (US EPA))	DT_{50} = 2.457 hours (0.205 days)	Yoshida M., Kodaka R., Fujisawa T. (2013) NNP-0120 RAR B.3
Vapour pressure	Pyriproxyfen	Measured	<1.33 x 10^{-5} Pa (<1.0 x 10^{-7} mmHg) at 22.81°C	Pesselman, R.L. (1989) NNP-91-0030

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

The vapour pressure of pyriproxyfen was experimentally determined to be <1.33 × 10^{-5} Pa at 22.81°C. The Henry's law constant was calculated as <7.37x10^{-2} Pa.m^3/mol at 23°C. These data show that pyriproxyfen is non-volatile.

Furthermore, pyriproxyfen is predicted to degrade very quickly in air with an AOPWIN (v1.92) estimated half-life of 2.5 hours (Yoshida, Kodaka and Fujisawa 2013; see section 11.3.1. for details). The DT50 of 2.5 hours is below the trigger of 2 days that is recommended by the FOCUS Working group on Pesticides (EC Document Reference SANCO/10553/2006 Rev 2 June 2008) as an identifier for substances of potential concern for long-range transport.

The FOCUS air working group developed a guidance methodology to determine the potential of a substance for atmospheric ozone depletion. The following issues are considered relevant:

1. The atmospheric life time of a substance should be long enough to transport the substance to the atmosphere;
2. The substance contains one or more of the following substituents: F, Cl of Br;

3. Substances containing N and S are relevant in stratospheric ozone depletion (e.g. N_2O);

Considering above, it can be concluded that as pyriproxyfen is not volatile, has a short atmospheric residence time, and does not contain F, Cl or Br, will not pose a hazard to the ozone layer.

12.1.2 Comparison with the CLP criteria

According to regulation (EC) 1272/2008 the classification criteria for substances are defined as:

A substance shall be classified as Hazardous to the Ozone Layer (Category 1) if the available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer.

Moreover, the Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures states that '*Any substances having an Ozone Depleting Potential (ODP) greater or equal to the lowest ODP (i.e. 0.005) of the substances currently listed in Annex I to Regulation (EC) No 1005/200989 should be classified as hazardous to the ozone layer (category 1).*'

For pyriproxyfen an ozone depletion potential was not determined and thus it cannot be determined if the criteria in regulation (EC) No 1005/200989 are fulfilled. However, pyriproxyfen is not volatile, has a short atmospheric residence time, and does not contain F, Cl or Br, and is not expected to pose a hazard to the ozone layer.

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

No classification proposed.

13 ADDITIONAL LABELLING

Not relevant.

14 REFERENCES

ECHA dissemination site (2022). https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/9704/4/9. Accessed 01-12-2022.

Pyriproxyfen is under evaluation for renewal of approval as a pesticide active substance according to Commission Regulation (EU) No 1107/2009. A reference list for the relevant studies from the RAR is included below:

Physical and chemical properties

Data point	**Author(s)**	**Year**	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**
CA 2.1/01	Pesselman, R.L.	1993a	Melting point determination of pyriproxyfen Company Report No. NNP-31-0054 Hazelton Wisconsin, Inc., USA GLP, Unpublished
CA 2.1/02	Isozaki, M.	2001	Determination of boiling point of pyriproxyfen

			Company Report No. NNP-0086 GLP, Unpublished
CA 2.2/01	Pesselman, R.L.	1989	Vapor pressure determination of Sumilarv Company Report No.NNP-91-0030 Hazelton Wisconsin, Inc., USA GLP, Unpublished
CA 2.3/03	Kimura, M.	2000a	Revision of 'Color of Sumilarv technical grade' Company Report No: NNP-0078 Not GLP, Unpublished.
CA 2.3/04	Kimura, M.	2000b	Revision of 'Physical state of Sumilarv technical grade' Company Report No: NNP-0079 Not GLP, Unpublished.
CA 2.5/02	Bates, M.L.	2006	Pyriproxyfen: evaluation of water solubility Company Report No. NNP-0105 Covance Laboratories, UK GLP, Unpublished
CA 2.6/01 CA 2.9/02	Bates, M.L.	2002	Pyriproxyfen: evaluation of physicochemical properties (EC Directive 91/414/EEC Annex II, Points 2.15, 2.7 and 2.11.1) Company Report No. NNP-0094 Covance Laboratories, UK GLP, Unpublished
CA 2.7/02	Bates, M. and Liney, P.	2005b	Pyriproxyfen: evaluation of the n-octanol/ water partition coefficient Company Report No. NNP-0103 Covance Laboratories, UK GLP, Unpublished
CA 2.8/01	Shigenaga, H.	1989	Dissociation constant of Sumilarv Company Report No. NNP-90-0022 Not GLP, Unpublished
CA 2.9/01 CA 2.11/01	Bates, M.	2001	Pyriproxyfen: determination of the physico-chemical properties (92/69/EEC tests A10, A14, A15) Company Report No. NNP-0091 Covance Laboratories, UK GLP, Unpublished

Toxicology and metabolism

Data point	**Author(s)**	**Year**	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**
CA 5.1.1/01	Anonymous	1998a	Metabolism of S-31183 in rats Company Report No: NNM-80-0001 Sumitomo Chemical Co Ltd GLP, Unpublished.
CA 5.1.1/02	Anonymous	1988b	Metabolism of S-31183 in rats (Tissue distribution study) Company Report No: NNM-80-0002 Sumitomo Chemical Co Ltd., Not GLP, Unpublished
CA 5.1.1/03	Anonymous	1993a	Metabolism of phenoxyphenyl-^{14}C-pyriproxyfen in rats (high-dose, ^{14}C-concentrations in tissues) Company Report No: NNM-30-0028 Sumitomo Chemical Co., Ltd GLP, Unpublished
CA 5.1.1/04	Anonymous	1993b	Metabolism of [pyridyl-2,6-^{14}C]pyriproxyfen in rats (pyridyl-^{14}C-labeled test compound, single oral administration at low- and high doses) Company Report No: NNM-30-0025 Sumitomo Chemical Co., Ltd GLP, Unpublished
CA 5.1.1/05	Anonymous	1995	Metabolism of pyriproxyfen 2. Comparison of *in vivo* metabolism between rats and mice J. Agric. Food Chem. 43, 2681-2686 Not GLP, Published
CA 5.2.1/01	Anonymous	1987a	Acute oral toxicity of S-31183 in rats Company Report No: NNT-70-0005 Sumitomo Chemical Co., Ltd GLP, Unpublished
CA 5.2.1/02	Anonymous	1987b	Acute oral toxicity of S-31183 in mice Company Report No: NNT-70-0014 Sumitomo Chemical Co., Ltd GLP unpublished
	Anonymous	1993	Addendum to the final report: Acute oral toxicity of S-31183 in mice Company Report No: NNT-70-0110 Sumitomo Chemical Co., Ltd GLP unpublished
CA 5.2.1/03	Anonymous	1986	Acute oral toxicity of S-31183 in dogs Company Report No: NNT-60-0012 Sumitomo Chemical Co., Ltd. GLP, Unpublished
CA 5.2.2/01	Anonymous	1987c	Acute dermal toxicity of S-31183 in rats Company Report No: NNT 70-0006

			Sumitomo Chemical Co., Ltd GLP, Unpublished
CA 5.2.2/02	Anonymous	1987d	Acute dermal toxicity of S-31183 in mice Company Report No: NNT-70-0015 Sumitomo Chemical Co., Ltd GLP, Unpublished
	Anonymous	1994	Amendment to the final report: Acute dermal toxicity of S-31183 in mice Company Report No: NNT-70-0111 Sumitomo Chemical Co., Ltd GLP, Unpublished
CA 5.2.3/01	Anonymous	1987	Acute inhalation toxicity of S-31183 in rats Company Report No: NNT-70-0022 Sumitomo Chemical Co Ltd., GLP, Unpublished
CA 5.2.3/02	Anonymous	1987e	Acute inhalation toxicity of S-31183 in mice Company Report No: NNT-70-0023 Sumitomo Chemical Co., Ltd GLP, Unpublished
	Anonymous	1995	Addendum to report: Acute inhalation toxicity of S-31183 in mice Company Report No: NNT-50-0131 Sumitomo Chemical Co., Ltd GLP, Unpublished
CA 5.2.4/01, CA 5.2.5/01	Anonymous	1987f	Primary eye and skin irritation tests with S-31183 in rabbits Company Report No: NNT-70-0004 Sumitomo Chemical Co., Ltd GLP, Unpublished
CA 5.2.6/01	Anonymous	1987g	Skin sensitization test with S-31183 in guinea pigs Company Report No: NNT-70-0003 Sumitomo Chemical Co., Ltd GLP, Unpublished
	Anonymous	1995	Amendment of Report: Skin sensitization test with S-31183 in guinea pigs Company Report No: NNT-50-0130 Sumitomo Chemical Co., Ltd GLP, Unpublished
CA 5.3.1/01	Anonymous	1988a	One-month oral toxicity study of S-31183 in rats Company Report No: NNT-80-0038 Sumitomo Chemical Co., Ltd GLP, Unpublished
CA 5.3.1/02	Anonymous	1987	Four-week oral toxicity study of S-31183 in dogs Company Report No: NNT-70-0013 Sumitomo Chemical Co., Ltd GLP, Unpublished
CA 5.3.2/01	Anonymous	1989	Sub-chronic toxicity study with S-31183 in rats Company Report No: NNT-91-0045

			Sumitomo Chemical Co Ltd., GLP Unpublished
CA 5.3.2/02	Anonymous	1988b	Six-month chronic oral toxicity study of S-31183 in rats Company Report No: NNT-80-0039 Sumitomo Chemical Co Ltd., GLP, Unpublished
CA 5.3.2/03	Anonymous	1988	Three-month oral toxicity study of S-31183 in dogs Company Report No NNT-80-0037 Sumitomo Chemical Co., Ltd. GLP, Unpublished
CA 5.3.2/04	Anonymous	1991	S-31183: Toxicity study by oral (capsule) administration to Beagle dogs for 52 weeks. Amended final report Company Report No. NNT-11-0081 LSR report no.: 91/0776 Life Science Research Limited, UK GLP, Unpublished
CA 5.3.2/05	Anonymous	1993	S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks (additional investigation) Company Report No: NNT-31-0102 Sumitomo Chemical Co., Ltd. Report No. NNT-31-0102 GLP, Unpublished
CA 5.3.3/01	Anonymous	1993	21-day dermal toxicity study in rats with S-31183 Company Report No: NNT-31-0094 Sumitomo Chemical Co Ltd., GLP, Unpublished
CA 5.3.3/02	Anonymous	1988	Sub-acute inhalation toxicity study of S-31183 in rats Company Report No: NNT 80-0031 Sumitomo Chemical Co Ltd., GLP, Unpublished
CA 5.4.1.1/01	Anonymous	1988	Reverse mutation test of S-31183 in bacterial systems Company Report No: NNT-80-0034 Sumitomo Chemical Co Ltd., GLP, Unpublished
CA 5.4.1.2/01	Anonymous	1989	*In vitro* chromosomal aberration test of pyriproxyfen in Chinese hamster ovary cells (CHO-K1) Company Report No. NNT-80-0054 Biochemistry and Toxicology Laboratory, Japan GLP, Unpublished
CA 5.4.1.3/01	Anonymous	1990	*In vitro* gene mutation test of S-31183 in V79 Chinese hamster cells Company Report No. NNT-90-0067 Biochemistry and Toxicology Laboratory, Japan GLP, Unpublished
CA 5.4.1.3/02	Anonymous	1988	Assessment of unscheduled DNA repair synthesis in mammalian cells after exposure to S-31183 Company Report No: NNT-91-0053 Sumitomo Chemical Co Ltd.,

CA 5.4.2/01	Anonymous	1991	Mouse micronucleus test on S-31183 Company Report No: NNT-11-0082 Sumitomo Chemical Co Ltd GLP, Unpublished
CA 5.5.1/01	Anonymous	1991a	Combined chronic toxicity and oncogenicity study in rats with S-31183 Company Report No: NNT-11-0085 Sumitomo Chemical Co Ltd., GLP, Unpublished
	Anonymous	1994	Addendum to the final report: Combined chronic toxicity and oncogenicity study in rats with S-31183 Company Report No. NNT-41-0112 Hazelton Washington, Inc., USA GLP, Unpublished
	Anonymous	1994	Amendment 1 & 2 to the final report: Combined chronic toxicity and oncogenicity study in rats with S-31183 Company Report No. NNT-41-0113 Hazelton Washington report no.: 343-214 Hazelton Washington, Inc., USA GLP, Unpublished
CA 5.5.1/02	Anonymous	1991b	Oncogenicity study in mice with S-31183 Company Report No: NNT-11-0084 Sumitomo Chemical Co Ltd., GLP, Unpublished
	Anonymous	1994	Amendment to the final report: Oncogenicity study in mice with S-31183 Company Report No. NNT-41-0117 Hazelton Laboratories America, Inc., USA GLP, Unpublished
	Anonymous	1994	Supplemental data and review of oncogenicity study with S-31183 (Sumilarv) in mice Company Report No. NNT-41-0116 Hazelton Laboratories America, Inc., USA GLP, Unpublished
CA 5.6.1/01	Anonymous	1991	A dietary 2-generation (1 litter) reproduction study of S-31183 in the rat. Company Report No: NNT-11-0087 Sumitomo Chemical Co Ltd GLP, Unpublished
CA 5.6.1/02	Anonymous	1988a	Study by orally administration of S-31183 to rats prior to and in the early stages of pregnancy Company Report No: NNT-81-0036 Sumitomo Chemicals Co Ltd., GLP, Unpublished
CA 5.6.2/01	Anonymous	1988b	Perinatal and postnatal study of S-31183 orally administered to rats Company Report No:NNT-80-0030 Sumitomo Chemical Co., Ltd. GLP, Unpublished

CA 5.6.2/02	Anonymous	1988c	Study by administration of S-31183 during the period of fetal organogenesis in rats Company Report No NNT 80-0029 Sumitomo Chemical Co., Ltd. GLP, Unpublished
CA 5.6.2/03	Anonymous	1988	Study of S-31183 by oral administration during the period of fetal organogenesis in rabbits Company Report No: NNT 80-0033 Sumitomo Chemical Co Ltd GLP, Unpublished
CA 5.7.1/01	Anonymous	2010	An oral (gavage) dose range-finding acute neurotoxicity study of pyriproxyfen T.G. in rats Company Report No. NNT-0181 WIL Research Laboratories, LLC, USA GLP, Unpublished
CA 5.7.1/02	Anonymous	2011a	An oral (gavage) acute neurotoxicity study of pyriproxyfen T.G. in rats Company Report No. NNT-0194 WIL Research Laboratories, LLC, USA GLP, Unpublished
CA 5.7.1/03	Anonymous	2011b	An 90-day oral dietary neurotoxicity study of pyriproxyfen T.G. in rats Company Report No. NNT-0202 WIL Research Laboratories, LLC, USA GLP, Unpublished
CA 5.8.2/01	Anonymous	2011	Pyriproxyfen: 4 week dietary immunotoxicity study in the female CD-1 mouse Company Report No. NNT-0204 Huntingdon Life Sciences, UK GLP, Unpublished
CA 5.8.3/01	Anonymous	2012a	A pubertal development and thyroid functions assay of pyriproxyfen T.G. administered orally in intact juvenile/peripubertal male rats Company Report No. NNT-0210 WIL Research Laboratories, LLC, USA GLP, Unpublished
CA 5.8.3/02	Anonymous	2012b	A pubertal development and thyroid function assay of pyriproxyfen T.G. administered orally in intact juvenile/peripubertal female rats Company Report No. NNT-0211 WIL Research Laboratories, LLC, USA GLP, Unpublished

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PYRIPROXYFEN (ISO); 2-(1-METHYL-2-(4-PHENOXYPHENOXY)ETHOXY)PYRIDINE; 4-PHENOXYPHENYL (*RS*)-2-(2-PYRIDYLOXY) PROPYL ETHER

Environmental fate and behaviour

Data point	**Author(s)**	**Year**	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**
CA 7.2.1.1/01	Katagi, T., Takahashi, N.	1989	Hydrolysis of S-31183 in buffered aqueous solutions at 50°C Sumitomo Chemical Co., Ltd. Report No. NNM-90-0013 GLP, Unpublished
CA 7.2.1.2/03	Ponte, M.	2015	Determination of the quantum yield of [^{14}C]pyriproxyfen in pH 7 buffer solution under artificial sunlight Sumitomo Chemical Co., Ltd, report No.: NNM-0087 PTRL West GLP, Unpublished
CA 7.2.2.1/01	Itoh, K., Tanoue, A., Matsuda, T.	1988	Biotic degradation of 1-(4-phenoxyphenoxy)-2-(2-pylidyloxy)propane (code name: S-31183) by activated sludge Sumitomo Chemical Co., Ltd. Report No. NNM-0064 GLP, Unpublished
CA 7.2.2.2/01	Adam, D.	2015	[^{14}C] Pyriproxyfen – aerobic mineralisation in surface water – simulation biodegradation test Sumitomo Chemical Co., Ltd, report No.: NNM-0086 Innovative Environmental Services (IES) Ltd GLP, Unpublished
CA 7.2.2.2/01a	Adam, D.	2017	Statement on procedural recoveries of [^{14}C] pyriproxyfen in aerobic mineralisation in surface water simulation biodegradation test Company report No. 20150070 Innovative Environmental Services (IES) Ltd, Switzerland Not GLP, Unpublished
CA 7.2.2.3/01	Lewis, C.J.	2000a	(^{14}C)-Pyriproxyfen: Degradation and retention in water-sediment systems Sumitomo Chemical Co., Ltd. Report No. NNM-0076 Covance Laboratories Ltd GLP, Unpublished
CA 7.2.2.4/01	Lewis, C.J.	2003	[^{14}C]Pyriproxyfen: Degradation in a water-sediment system in the light. Sumitomo Chemical Co., Ltd., Unpublished report No.: NNM-0081 Covance Laboratories Ltd GLP, Unpublished **Study provided at the request of the RMS** PREVIOUSLY SUBMITTED

Data point	**Author(s)**	**Year**	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**
CA 7.2.2.3/03	Cooke, J.	2016b	Pyriproxyfen: kinetic assessment of water/sediment studies Sumitomo Chemical Co., Ltd, report No.: NNM-0096 JSC International Limited Not GLP, Unpublished
CA 7.3/04	Yoshida, M., Kodaka, R., Fujisawa, T.	2013	Stability of pyriproxyfen in air (calculation by Atkinson's method) Sumitomo Chemical Co., Ltd, report No.: NNP-0120 Not GLP, Unpublished

Ecotoxicology

Data point	**Author(s)**	**Year**	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**
CA 8.1.4/01	Anonymous	2012	Pyriproxyfen – Amphibian Metamorphosis Assay with African Clawed Frog (Xenopus laevis) Following OPPTS Test Guideline 890.1100 and OECD Test Guideline 231 Company Report No.: NNW-0211 Smithers Viscient, USA GLP, Unpublished
CA 8.2.1/01	Anonymous	1989a	Acute flow-through toxicity of Sumilarv to rainbow trout (Salmo gairdneri). Sumitomo Chemical Co., Ltd. Unpublished report No.: NNW-91-0035
CA 8.2.1/02	Anonymous	1989b	Acute flow-through toxicity of Sumilarv to bluegill (Lepomis macrochirus) Company Report No.: NNW-91-0034 Analytical Bio-Chemistry Laboratories, Inc.,USA GLP, Unpublished
CA 8.2.1/05	Anonymous	1991	Acute flow-through toxicity of Sumilarv T.G. to the Sheepshead minnow (Cyprinidon variegatus) Company Report No.: NNW-11-0070 Analytical Bio-Chemistry Laboratories, Inc., USA GLP, Unpublished

Data point	**Author(s)**	**Year**	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**
CA 8.2.2/02	Anonymous	1991	Early life-stage toxicity of Sumilarv technical to the rainbow trout (Oncorhynchus mykiss) under flowthrough conditions Company Report No.: NNW-11-0062 ABC Laboratories, Inc., USA GLP, Unpublished
CA 8.2.1/01b CA 8.2.2/03	Anonymous	2016	Derivation of endpoints (EC10 and EC20 values) for fish early lifestage toxicity study with pyriproxyfen. Sumitomo Chemical Co., Ltd. Unpublished report No.: NNW-0248
CA 8.2.2.2/01 CA 8.2.2.1/01	Anonymous	2007	Pyriproxyfen: Full Life Cycle Toxicity Test with Medaka (Oryzias tatipes) under Flow- Through Conditions Company Report No.: NNW-0181 Springborn Smithers Laboratories (Europe), Switzerland GLP, Unpublished
CA 8.2.3/01 CA 8.2.4.1/01	Burgess, D.	1989	Acute flow-through toxicity of Sumilarv to Daphnia magna Company Report No.: NNW-91-0036 Analytical Bio-Chemistry Laboratories, Inc.,USA GLP, Unpublished
CA 8.2.3/06 CA 8.2.4.2/01	Dionne, E.	1998	Sumilarv T.G. – Acute Toxicity to Eastern Oyster (Crassostrea virginica) Under Flow-Through Conditions Company Report No.: NNW-0138 Springborn Laboratories Inc., USA GLP, Unpublished
CA 8.2.3/07 CA 8.2.4.2/01	Sousa, J.V.	1999	Sumilarv T.G. – Acute Toxicity to Mysids (Mysidopsis bahia) Under Flow-Through Conditions Company Report No.: NNW-0139 Springborn Laboratories Inc., USA GLP, Unpublished
CA 8.2.4/01a CA 8.2.5.1/01a	Blakemore, G.C. Butzlaff, T.S. Stuerman, L.	1992	Chronic toxicity of ^{14}C-Sumilarv to Daphnia magna under flow-through test conditions Company Report No.: NNW-21-0075 ABC Laboratories, Inc., USA GLP, Unpublished
CA 8.2.4/01b CA 8.2.5.1/01b	Lewis, G.	2016	Derivation of endpoints (EC10 and EC20 values) for Daphnia magna chronic toxicity study with pyriproxyfen. Sumitomo Chemical Co., Ltd. Unpublished report No.: NNW-0247

Data point	**Author(s)**	**Year**	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**
CA 8.2.4/02 CA 8.2.5.1/02	Rhodes, J.E. Bucksath, J.D.	1996	Chronic Toxicity of PYPAC and 4'-OHPyr to Daphnia magna Under Flow-Through Conditions Company Report No.: NNW-0127 ABC Laboratories, Inc., USA GLP, Unpublished
CA 8.2.4/03 CA 8.2.5.1/03	Shaw, A.C.	2015a	4'-OH-Pyr – Full Life-Cycle Toxicity Test with Water Fleas, Daphnia magna, Under Flow-Through Conditions Following OECD Guideline #211 Company Report No.: NNW-0238 Smithers Viscient, USA GLP, Unpublished
CA 8.2.4/06 CA 8.2.5.2/01	Hagino, S., Matsuda T.	1992a	Effect of pyriproxyfen on growth of Asellus hilgendorfii (Crustacea). Sumitomo Chemical Co., Ltd. Unpublished report No.: NNW-21-0081
CA 8.2.4/07 CA 8.2.5.2/02	Hagino, S., Matsuda T.	1992d	Effect of pyriproxyfen on reproduction of Asellus hilgendorfii (Crustacea). Sumitomo Chemical Co., Ltd. Unpublished report No.: NNW-21-0082
CA 8.2.4/08 CA 8.2.5.2/03	Hagino, S., Matsuda T.	1992c	Effect of pyriproxyfen on growth of Tigropius japonicus. Sumitomo Chemical Co., Ltd. Unpublished report No.: NNW-21-0085
CA 8.2.4/09 CA 8.2.5.2/04	Hagino, S., Matsuda T.	1992b	Effect of pyriproxyfen on reproduction of Tigropius japonicus. Sumitomo Chemical Co., Ltd. Unpublished report No.: NNW-21-0084
CA 8.2.4/10 CA 8.2.5.2/05	10 Kagoshima, M., Kobayashi, H., Sato, T., Takimoto Y.	1995	Effect of pyriproxyfen on reproduction of Daphnia pulex. Sumitomo Chemical Co., Ltd. Unpublished report No.: NNW-50-0120
CA 8.2.4/11a CA 8.2.5.2/06	Machado, M.W.	1995	Sumilarv (pyriproxyfen) - Chronic toxicity to mysids (Mysidopsis bahia) under flowthrough test conditions Company Report No.: NNW-51-0119 Springborn Laboratories, Inc., USA GLP, Unpublished

Data point	**Author(s)**	**Year**	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**
CA 8.2.4/11b CA 8.2.5.2/06b	Lewis, G.	2016	Derivation of endpoints (EC10 and EC20 values) for Mysidopsis bahia chronic toxicity study with pyriproxyfen. Sumitomo Chemical Co., Ltd. Unpublished report No.: NNW-0249
CA 8.2.4/12 CA 8.2.5.2/07	Biester, M.A.	2011	Pyriproxyfen: Chronic Toxicity Test with Mayfly Nymphs (Cloeon dipterum) Under Semi-Static Conditions Company Report No.: NNW-0209 Smithers Viscient AG, Switzerland GLP, Unpublished
CA 8.2.4/13 CA 8.2.5.3/01	Putt, A.E.	2003	Pyriproxyfen – The full life-cycle toxicity to midge (Chironomus riparius) under static conditions Company Report No.: NNW-0157 Springborn Smithers Laboratories, USA GLP, Unpublished
CA 8.2.4/14 CA 8.2.5.4/01	Picard, C.	2015	42-Day Toxicity Test Exposing Freshwater Amphipods (Hyalella azteca) to Pyriproxyfen Applied to Sediment Under Static-Renewal Conditions Following EPA Test Methods Company Report No.: NNW-0243 Smithers Viscient, USA GLP, Unpublished
CA 8.2.5/01 CA 8.2.6.1/01	Blasberg, J.W. Hicks, S.L. Cramer, D.L.	1991	Acute toxicity of pyriproxyfen to Selenastrum capricornutum Prinz Company Report No.: NNW-11-0068 ABC Laboratories, Inc., USA GLP, Unpublished
CA 8.2.5.2/08	Roessink, I.	2018	Chronic effects of the insecticide pyriproxyfen to three aquatic macroinvertebrates Company Report No. NNW-0259 Alterra, The Netherlands GLP, Unpublished
CA 8.2.6/01 CA 8.2.6.1/02	Hoberg, J.R.	1996	Pyriproxyfen - Toxicity to duckweed, Lemna gibba Company Report No.: NNW-0126 Springborn Laboratories, Inc., USA GLP, Unpublished

Data point	**Author(s)**	**Year**	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**
CA 8.2.7/01a CA 8.2.2.3/01a	Anonymous	1993	Uptake, depuration and bioconcentration of ^{14}C-pyriproxyfen by bluegill sunfish Company Report No.: NNM-31-0027 Batelle Columbus Division, USA GLP, Unpublished
CA 8.2.7/01b CA 8.2.2.3/01b	Anonymous	1994	Characterization of ^{14}C-residues in bluegill sunfish treated with ^{14}Cpyriproxyfen Company Report No.: NNM-41-0031 Batelle Metabolism Chemistry, USA GLP, Unpublished
CA 8.2.7/01c CA 8.2.2.3/01c	Anonymous	1999	Calculation of ^{14}Cpyriproxyfen clearance time (CT90, CT95) in bluegill sunfish Company Report No.: NNM-0061 Environmental Health Science Laboratory, Japan Not GLP, Unpublished
CA 8.2.7/02a CA 8.2.2.3/02a	Anonymous	1993a	Bioaccumulation of 1-(4- Phenoxyphenoxy)- 2-(2-pyridyloxy)propane(S-31183) in Fish Company Report No.: NNR-90-0020 Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Japan GLP, Unpublished
CA 8.2.7/02b CA 8.2.2.3/02b	Anonymous	1993b	Excretion Study of 1-(4-Phenoxyphenoxy)-2-(2-pyridyloxy)propane(S-31183) Company Report No.: NNR-90-0020-1 Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Japan GLP, Unpublished

15 ANNEXES

The study summaries from the RAR of pyriproxyfen have been included in Annex I. Confidential Annex II contains the references indicated above as anonymous.