Committee for Risk Assessment

RAC

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

Adopted

14 March 2024

14 March 2024

CLH-O-0000007433-76-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted on **14 March 2024** by **consensus** an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **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**

Rapporteur, appointed by RAC: **Mihaela Pribu**

Co-Rapporteur, appointed by RAC: **Riitta Leinonen**

Administrative information on the opinion

Netherlands on **8 February 2023** has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **13 March 2023**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **12 May 2023**.

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The following table provides a summary of the Current Annex VI entry, Dossier submitter proposals, RAC opinions and potential Annex VI entries if agreed by the Commission.

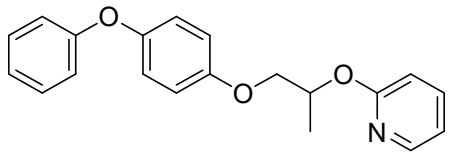
Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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	
RAC opinion	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=1000	
Resulting Annex VI entry if agreed by 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-1000	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Pyriproxyfen (ISO); 2-(1-methyl-2-(4-phenoxyphenoxy)ethoxy)pyridine; 4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propyl ether (structure below) is a broad-spectrum pesticide which is used to protect cotton crops against whitefly and recently found also to be effective in other crops.



It is registered as a biocidal active substance under Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products (PT19) and is commonly used directly on pets to control fleas and ticks. Pyriproxyfen products can come in many forms, including liquids, granules, dusts, and pellets. Other products are used in aquatic settings like birdbaths and ponds. Some materials are infused with pyriproxyfen, such as pet flea collars.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

No classification for physical hazards was proposed by the DS.

Pyriproxyfen is a solid substance and consequently the DS concluded that hazard classes flammable gases, oxidising gases, gases under pressure, flammable liquids, pyrophoric liquids, and oxidising liquids were not applicable.

Explosives

After theoretical assessment of the chemical structure of pyriproxyfen, no explosive groups were found, the oxygen balance was less than -200 (-231.5 %). Additionally, a differential scanning calorimetry (DSC) technique was applied, and the exothermic decomposition energy did not have an exothermic peak >500 °C (~480 °C) (Bates, 2001, NNP-0091). Consequently, the DS concluded that the criteria for classification as explosive were not met.

Flammable solids

Pyriproxyfen was tested for its flammable properties in an EC A.10 test (Bates, 2001, NNP-0091). 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, the DS concluded that the criteria for classification as flammable solid were not met.

Self-reactive substances

No study was available. There are no chemical groups present in the molecule associated with explosive or self-reactive properties. Consequently, the DS concluded that the criteria for

classification as a self-reactive substance were not met.

Pyrophoric solids

No study was available. Pyriproxyfen has been handled extensively in air and has never self-ignited. Consequently, the DS concluded that the criteria for classification as a pyrophoric solid were not met.

Self-heating substances

The melting point of pyriproxyfen is 48-50 °C. According to the CLP Criteria substances with a low melting point (<160 °C) should not be considered self-heating since the melting process is endothermic and the substance-air surface is drastically reduced. Consequently, the DS concluded that the criteria for classification as self-heating substance were not met.

Substances which in contact with water emit flammable gases

In an EC A.12 study (Bates, M.L. 2002 NNP-0094), pyriproxyfen did not produce flammable gases when in contact with water. Additionally, experience in handling and use shows that the substance does not react with water to emit flammable gases. Consequently, the DS concluded that the criteria for classification as a substance which in contact with water emits flammable gasses were not met.

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 DS concluded that the criteria for classification as oxidising solid were not met.

Organic peroxides

The DS concluded that the criteria for classification as organic peroxide were not met. The definition for organic peroxides, liquid or solid organic substances which contain the bivalent -O-O- structure and may be considered derivatives of hydrogen peroxide where one or both of the hydrogen atoms have been replaced by organic radicals, were not met.

Corrosive to metals

No study was available. The test substance is a solid which may become liquid during transport (melting point 48.0-50.0 °C). 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 was not required. The DS concluded that the criteria for classification as corrosive to metals was not met.

Comments received during consultation

No comments were received in the consultation.

Assessment and comparison with the classification criteria

Explosives

RAC concludes that the criteria for classification as explosive are not met due to a lack of chemical groups associated with explosive properties in the molecule (CLP Annex I, 2.1.4.3 a-c).

Flammable solids

RAC concludes that the criteria for classification as flammable solid are not met, based on an EC A.10 study concluding that the substance is not highly flammable (CLP Annex I, 2.7.2).

Self-reactive substances

RAC concludes that the criteria for classification as self-reactive substance are not met due to lack of chemical groups associated with explosive or self-reactive properties (CLP Annex I, 2.8.4.2 a).

Pyrophoric solids

RAC concludes that the criteria for classification as pyrophoric solid are not met, based on experience in manufacturing and handling (CLP Annex I, 2.10.4.1).

Self-heating substances

The melting point of pyriproxyfen is 48-50 °C. CLP Guidance (2.11.4.2) states that substances with a low melting point (<160 °C) should not be considered for classification in this hazard class. The criterion is only applicable if the substance is completely molten up to this temperature. RAC concludes that the criteria for classification as self-heating substance are not met.

Substances which in contact with water emit flammable gases

Experience in handling and use shows that the substance does not react with water to emit flammable gases which fulfils the screening criteria (CLP Annex I 2.12.4.1 (b)). RAC concludes that the criteria for classification as substance which in contact with water emits flammable gases are not met not.

Oxidising solids

Pyriproxyfen contains oxygen which is only bonded to carbon fulfilling the screening criteria (CLP Annex I 2.14.4.1 (b)). RAC concludes that the criteria for classification as oxidising solid are not met.

Organic peroxides

Pyriproxyfen does not contain a peroxide group (-O-O) and thus fulfils the screening procedure for no classification. RAC concludes that the criteria for classification as organic peroxide are not met.

Corrosive to metals

The test substance is a solid which may become liquid during transport (melting point 48.0-50.0 °C). However, the substance does not contain acid or basic functional groups, nor halogens, nor form complexes with metals and consequently fulfils the screening criteria presented in the CLP Guidance 2.16.4.1. Consequently, RAC concludes that the criteria for classification as corrosive metals are not met.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity

The dossier submitter (DS) provided (in the CLH report, table 25) the results of three acute oral toxicity studies, one in rats (Draft Assessment Report [DAR], Vol. 3 B.6.2.1 - CA 5.2.1/01 (1987a)), and one in mice (DAR, Vol. 3. B.6.2.1 - CA 5.2.1/02 (1987b)) and another in a dog (DAR, Vol. 3. B.6.2.1 - CA 5.2.1/03 (1986).

The studies were considered valid, scientifically acceptable, and appropriate for the assessment of acute oral toxicity. The oral LD_{50} value was > 5000 mg/kg bw in male and female rats as well as in male and female mice. The study with the dog (1/sex) was range-finding and did not allow LD_{50} determination as the animal survived the top dose of 5000 mg/kg bw.

No human data are available.

According to the DS, since the oral LD_{50} values of pyriproxyfen in both rats and mice are > 2000 mg/kg bw, classification for acute oral toxicity according to CLP is not required.

Acute dermal toxicity

The DS provided (in the CLH report, table 28) the results of two acute dermal toxicity studies performed in 1987 in rats (DAR, Vol. 3 B.6.2.2 - CA 5.2.2/01) and in mice (DAR, Vol. 3. B.6.2.2 - CA 5.2.2/02). The studies were considered valid, scientifically acceptable and appropriate for the assessment of acute dermal toxicity. The dermal LD_{50} value of pyriproxyfen was > 2000 mg/kg bw. No human data are available.

According to the DS, since the oral LD_{50} values of pyriproxyfen in rats and mice are > 2000 mg/kg bw, classification for acute dermal toxicity according to CLP is not required.

Acute inhalation toxicity

The DS provided (in the CLH report, table 31) the results of two acute inhalation toxicity studies performed in 1987 in rats (DAR, Vol. 3, B.6.2.3 - CA 5.2.3/01) and mice (DAR, Vol. 3, B.6.2.3 - CA 5.2.3/02). Both studies were considered valid, scientifically acceptable and appropriate for the assessment of acute inhalation toxicity. The acute inhalation LC_{50} value in both male and female rats and mice was >1.3 mg/L. This was the highest technically achievable concentration.

According to the DS, since no mortality was observed up to the highest attainable dust aerosol concentration of 1.3 mg/L in either species, a classification for acute inhalation toxicity according to CLP is not required. No data are available to indicate a mechanism of corrosivity. No human data are available.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Acute oral toxicity

The acute oral toxicity study (CA 5.2.1/01(1987a)) was performed on five rats, Sprague-Dawley/sex/group who received pyriproxyfen by gavage at dose levels of 1000, 2500, 5000 mg/kg bw (single dose). There were no mortalities or clinical signs of toxicity at none of the administered doses. Decreased motor activity was observed in males at the 2500 mg/kg bw level and soft faeces and diarrhoea were observed in males and females given 5000 mg/kg bw.

A significant decrease in body weight and body weight gain was noted among the females given 5000 mg/kg bw throughout the observation period.

The results of the histopathology examination noted changes in white substance in the urinary bladder and uterine horn distended with fluid in some animals in all groups including control animals

In the acute oral toxicity study in (CA 5.2.1/02(1987b)), five ICR mice /sex/group received pyriproxyfen by gavage at dose levels of 1000, 2000, 5000 mg/kg bw (single dose).

The acute oral toxicity study (CA 5.2.1/03(1986)) was performed on one Beagle dogreceived pyriproxyfen in gelatinous capsules at dose levels of 500, 1500, 5000 mg/kg bw (single dose). No mortality was observed at any dose administered. The study was performed in accordance with OECD TG 401 (1987) with the deviation that only a single animal was used, instead of 5 animals. The results of the study are not relevant.

As the available oral LD_{50} values are greater than 5000 mg/kg bw, RAC agrees with the DS's proposal of **no classification for acute toxicity via the oral route**.

Acute dermal toxicity

In the acute dermal toxicity study in SD rats (CA 5.2.2/01(1987c)), pyriproxifen as semiocclusive patch (24 hours on a skin area of 30 cm^2) was applied to the skin of five animals/sex/group at dose levels of 0, 2000 mg/kg bw for 24 hours. No mortality occurred and the LD_{50} was >2000 mg/kg bw.

In the acute dermal toxicity study in ICR mice (CA 5.2.2/02 (1987d)), pyriproxifen as semiocclusive patch (24 hours on a skin area of 4.5 cm^2) was applied to the skin of five animals/sex/group at dose levels of 0, 2000 mg/kg bw for 24 hours. There were no mortalities, no clinical signs of toxicity and no gross findings at necropsy other than white substance in the urinary bladder which was seen in 2 animals in the control group and uterine horn distension with fluid which was observed in 3 females of the 2000 mg/kg bw group.

As the dermal LD_{50} is >2000 mg/kg bw, RAC agrees with the DS's proposal of **no classification for acute toxicity via the dermal route**.

Acute inhalation toxicity

In the acute inhalation toxicity study in SD rats (CA 5.2.3/01(1987)), five males and five females were exposed (whole-body) to pyriproxyfen (MMAD 0.86/0.75 µm(mist)GSD 1.35/1.55) for 4 hours at two exposure concentrations of 0.6 mg/L and 1.3 mg/L.

Toxicity of pyriproxyfen was shown by salivation in two from five males and one from five females at 1.3 mg/L. Urinary incontinence was noticed in two from five females four hours after the beginning of exposure. Body weight gain was decreased slightly in males in the 1.3 mg/L dose group three days after the end of exposure with recovery at day 7. The histopathological examination showed brown points on the lung surface in one from five males in the 0.6 mg/L dose group and one from five females in the vehicle dose group. White substance in the lumen of the urinary bladder was observed in some males and uterine horn distended with fluid was observed in some females. However, these changes were noted among all groups without a dose-relationship.

In the lungs some minimal changes were observed in one or two males or females of each group.

In the acute oral and dermal toxicity studies in rats, no mortality and no clinical signs, effects on body weight or pathological changes were observed at the highest dose level of 2000 mg/kg bw.

No mortality or treatment-related effects were observed in the acute inhalation toxicity study in rats at doses of 0.6 or 1.3 mg/L.

In the acute inhalation toxicity study in ICR mice (CA 5.2.3/02(1987e)), five males and five females were exposed (whole-body) to pyriproxyfen (MMAD 0.86/0.75 µm (mist) GSD 1.35/1.55) for 4 hours at two exposure concentrations (0.6 mg/L and 1.3 mg/L).

No mortality was noted. No significant effect on body weight gain was observed. Symptoms at the highest dose included irregular respiration in one to two male or female animals 2 hours after the start of the experimental study.

The gross pathology revealed some pulmonary lesions including minimal agonal haemorrhage that are considered incidental (one male in the vehicle control group and one female in the high dose group).

As the 4-hour LC_{50} is >1.3 mg/L, RAC agrees with the DS's proposal for **no classification for acute toxicity via the inhalation route**.

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

Summary of the Dossier Submitter's proposal

For assessment of STOT SE the DS has referred to studies on acute toxicity and acute neurotoxicity described in the DAR (Volume 1, section 2.6.2 and 2.6.7 and to Volume 3, section B.6.2 and B.6.7) which are summarised in the section above.

In the acute oral toxicity study in mice (CA 5.2.1/02 (1987b), performed at 1000, 2000, 5000 mg/kg bw (single dose administrated via gavage), 2/5 males died within 2 days after treatment at 2000 mg/kg bw, whereas 2/5 males died within 2 days and 1/5 female on day 1 after treatment at 5000 mg/kg bw.

Two neurotoxicity rat studies were performed in rats (CLH report, table 61), a dose –range finding study (CA 5.7.1/01 (2010) and an acute neurotoxicity study (CA 5.7.1/02 (2011a)). Under the conditions of the first study, no specific toxicological findings were observed up to the highest dose level of 2000 mg/kg bw, which was considered to be a suitable maximum dose for the definitive acute oral neurotoxicity study. In the second study, the DS mentioned a decrease in motor activity at 1000 and 2000 mg/kg bw but concluded that this effect is likely a result of the general toxicity based on the clinical signs rather than a specific neurotoxic effect. No effects on neurobehaviour were observed in the functional observation battery (FOB), neurohistopathology, brain weights or brain dimensions in either sex.

Therefore, no relevant effects indicating a severe toxicity were observed after oral, dermal or inhalation exposure, in the mouse study or in the range-finding study for the acute neurotoxicity study. There were no signs related to respiratory tract irritation or narcotic effects. No human data are available.

According to the DS, no specific target organ toxicity, even in the absence of mortality, was observed after single dosing of animals thus classification of pyriproxyfen for STOT SE according to CLP is not required.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Acute neurotoxicity study in rats (CA 5.7.1/02 (2011a))

Pyriproxyfen was administrated by gavage, at doses of 0, 300, 1000, 2000 mg/kg bw in SD rats (12/sex/group). Approximately 24 hours following dose administration animals showed an *unkempt appearance* at 2000 mg/kg bw group (1/12 males and 6/12 females) at the detailed

physical examination A value of the NOAEL for general toxicity was set as being 1000 mg/kg bw. The general observation of male rats noted a decrease in total and ambulatory motor activity counts at 1000 and 2000 mg/kg bw. The NOAEL for acute neurotoxicity was 300 mg/kg bw.

In the acute neurotoxicity study, a decrease in total and ambulatory motor activity counts was observed in male rats only. Detailed quantification of observations from the acute neurotoxicity study in rats are presented in the table below.

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
- 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, while the decrease in motor activity was only observed in males, which was not considered severe enough for classification for STOT-SE. Since no significant and/or severe toxic effects of relevance to human health, that there were no significant acute toxicity effects via the oral, dermal or inhalation routes, RAC concluded that **no classification for pyriproxyfen for STOT SE in Category 1 or 2 is warranted**.

Since neither significant toxic effects nor narcotic effects and respiratory tract irritation were observed in the appropriate studies in experimental animals RAC is of the opinion that pyriproxyfen does not warrant classification for specific target organ toxicity – single exposure.

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 warranted.**

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The potential of pyriproxyfen to induce skin irritation was investigated in a rabbit study performed according to OECD TG 404 and GLP (CLH dossier, table 34). The study deviated from the OECD TG 404 because an occlusive dressing was used during the application of pyriproxyfen (DAR, Volume 3, section B.6.2.4 - CA 5.2.4/01(1987f). No irritation reactions were observed in the skin

of any animal at any observation time (24-72 hours). Based on these data DS concluded that pyriproxyfen does not require classification for skin corrosion/irritation.

The DS proposed no classification based on an *in vivo* skin irritation study in rabbits with occlusive dressing showing no irritation. No human data are available.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

In the *in vivo* skin irritation study (CA 5.2.4/01 (1987f)), the individual and mean scores for erythema and edema at 1, 24, 48 and 72 hours were 0 for all animals. In the acceptable skin irritation/corrosion study in rabbits the CLP criteria for skin irritation of a mean score of ≥ 2.3 for erythema/eschar or for oedema were not observed in any of the tested animals, therefore **RAC concludes that pyriproxyfen does not warrant classification for skin corrosion/irritation.**

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The eye irritancy of pyriproxyfen was investigated in a rabbit study performed according to OECD TG 405 and GLP (CLH dossier, table 37). Pyriproxyfen was administered as a 0.1 g dose into the conjunctival sac in one eye each of 3/sex rabbits NZW; (DAR, Volume 3, section B.6.2.5 - CA 5.2.5/01(1987f). The treated eyes of the animals remained unwashed after application. All animals were subjected to observations at 1, 24, 48 and 72 hours after application to verify the ocular response in the cornea, iris and conjunctiva. Without washing, the mean irritation score (24, 48, 72 hours) was 0.33 for chemosis. With washing, the mean irritation score (24, 48, 72 hours) in all rabbits was 0. Based on results of this study DS concluded that pyriproxyfen does not require classification for skin corrosion/irritation.

No human data are available.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

In the *in vivo* eye irritation study (CA 5.2.5/01 (1987f)), pyriproxyfen was applied without washing into the eyes of 3 rabbits NZW/sex. No conjunctival chemosis or conjunctival erythema nor corneal opacity were observed. The individual mean scores (24, 48 and 72 h) were 0 for all of eyes effects. The results of the study noted that were observed some reactions at pyriproxyfen but all of them were reversible within 72 hours.

Treated-unrinsed eyes (6 animals)

Scores observed after	1 hour	24 hours	48 hours	72 hours

Cornea/opacity	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0 (0)	0, 0, 0, 0, 0, 0 (0)	0, 0, 0, 0, 0, 0 (0)
Iris	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0 (0)	0, 0, 0, 0, 0, 0 (0)	0, 0, 0, 0, 0, 0 (0)
Conjunctiva redness	1, 1, 1, 1, 1, 1	0, 0, 0, 0, 1, 1 (0.33)	0, 0, 0, 0, 0, 0 (0)	0, 0, 0, 0, 0, 0 (0)
Conjunctiva chemosis	1, 1, 1, 1, 2, 2	0, 0, 0, 0, 0, 1 (0.17)	0, 0, 0, 0, 0, 0 (0)	0, 0, 0, 0, 0, 0 (0)

As the mean scores for the specific ocular effects are not exceeding the CLP criteria for classification in Category 2. Only slight conjunctival redness and chemosis were observed, but the average scores were < 2 (i.e., the relevant average score for conjunctival redness and oedema). Therefore, **RAC concludes that classification of pyriproxyfen for eye damage/irritation is not warranted.**

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS provided results of one skin sensitisation study with pyriproxyfen (CLH dossier, table 43), i.e. a Guinea pig maximisation test (GPMT) performed in 1987. This was not entirely in line with the OECD TG 406, where it is indicated that the highest non-irritant dose should be used; however, it is in line with the article by Magnussen and Kligman (1969).

For this reason, the DS considers the concentration of 25% petrolatum ointment as an adequate concentration for the challenge treatment. Taking in account that the sensitisation rate in the GPMT was 0%, the DS concluded that classification of pyriproxyfen for skin sensitisation according to CLP is not required.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The GPMT (CA5.2.6/01 (1987g)) used 20 negative control and 20 treated male Guinea pigs (Dunkin/Hartley). The intradermal induction concentration was 0.5% pyriproxyfen in corn oil; no irritation was observed. Topical application was conducted with 25% pyriproxyfen in petrolatum.

Dose levels for this study were based on the results of a range-finding study using 0.1, 0.25, 0.5 and 1% for intradermal injections and 25% for topical applications. The test substance concentration of 25% was stated to be the maximum application concentration for sensitisation in general (Magnussen and Kligman, J. Invest. Derm 1969). In this paper the following is stated: "*challenge by topical application. Provided there is no irritation, solids are incorporated in petrolatum at 25% concentration and liquids are used as is*."

Slight erythema was noted at 0.5% and slight erythema and swelling at 1.0% in the intradermal application. No skin reaction was noted after 25% topical application.

RAC noted that a test conducted with non-irritant concentrations of pyriproxyfen greater than 25% was not presented in the dossier. RAC concluded that **no classification of pyriproxyfen for skin sensitisation is warranted based on inconclusive data**.

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

Summary of the Dossier Submitter's proposal

There were no data indicating that pyriproxyfen has induced adverse effects in humans as a result of long-term exposure. The DS provided the results of several relevant experimental studies in rats, mice and dogs (CLH report, table 64) . The main target organs were the liver and kidney, but effects were mild and not considered sufficient for classification.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

According to CLP regulation, substances are classified for target organ toxicity STOT RE 1 if they 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.

Classification with STOT RE 1 is triggered by the occurrence of significant toxic effects in experimental animals after exposure at or below the guidance values, dependent upon the route of administration and time of exposure.

For a 90-day repeated-dose study guidance values provided in Table 3.9.2 (CLP regulation, Annex I) are equal to:

Oral: C ≤ 10 mg/kg bw/day

Dermal: C ≤ 20 mg/kg bw/day

Substances are classified in STOT RE 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 (CLP regulation, Annex I, Section 3.9.2.1).

Guidance values for STOT RE 2 referring to effects seen in a standard 90-day toxicity study conducted in rats (CLP regulation, Annex I, Table 3.9.3):

Oral: 10 < C ≤ 100 mg/kg bw/day

Dermal: 20 < C ≤ 200 mg/kg bw/day

Using Haber's rule for comparison with CLP classification criteria for STOT RE 2 the following guidance values were adopted for the oral exposure:

10 < C ≤ 100 mg/kg bw/day, duration 90 days/3 months/13 weeks

7.7 < C ≤ 77 mg/kg bw/day, duration 17 weeks

2.5 < C ≤ 25 mg/kg bw/day, duration 52 weeks

1.7 < C ≤ 17 mg/kg bw/day, duration 78 weeks

1.25 < C ≤ 12.5 mg/kg bw/day, duration 104 weeks

0.05 < C ≤ 0.25 mg/L/6 h/day, duration 365 days

0.03 < C ≤ 0.13 mg/L/6 h/day, duration 730 days

For dermal route

C ≤ 20 mg/kg bw/day, duration 90 days

C ≤ 86 mg/kg bw/day, duration 21 days

Since there are no data on specific target organ toxicity due to repeated exposure in humans this hazard class has to be assessed based on data derived from the acceptable animal studies. As a summary of the performed studies on pyriproxyfen, liver injuries (hypertrophy) in the sub-acute studies and observations in the sub chronic studies were hepatocellular injury, hepatobiliary effects, cholestasis in rats and dogs and secondary renal failure in the rat. In addition, in all the studies performed by dietary exposure, in all species presented slight changes in haematological parameters.

According with the Guidance on the Application of the CLP Criteria, Version 5.0 - July 2017, Part. 4, 3.9.2. Classification of substances for STOT-RE, in Annex 1: 3.9.2.5. .... "*The standard animal studies in rats or mice that provide this information are 28 day, 90 day or lifetime studies (up to 2 years) that include haematological, clinico-chemical and detailed macroscopic and microscopic examination to enable the toxic effects on target tissues/organs to be identified. Data from repeat dose studies performed in other species shall also be used, if available. Other long-term exposure studies, such as on carcinogenicity, neurotoxicity or reproductive toxicity, may also provide evidence of specific target organ toxicity that could be used in the assessment of classification*."

There were no human studies available or results in experimental studies which could support classification of pyriproxyfen in Category 1.

In a 28-day rat study (CA 5.3.1/01, (1998a)) by dietary exposure at the concentration of 1000 ppm (equivalent to 95.8 mg/kg bw/day in males and 97.6 mg/kg bw/day in females), the adverse effects on liver were below the (extrapolated) GV for STOT RE category 2, 300 mg/kg bw/day, respectively.

However, the adverse effects observed at this dose level were limited to a marginal increase in serum albumin, globulin concentrations and changes in A: G ratio in males. Cell damage and histopathological evidence for effects on the liver were not presented at this dose level. The results in the liver reflect changes in liver function and these are not considered to be relevant for classification of pyriproxyfen as STOT RE.

Studies of 90-days duration did not report relevant effects for classification of pyriproxyfen as STOT RE. The 6-month study in mice and 1-year study in dog revealed toxic effects only at dose levels above the GV relevant for classification. The (extrapolated) GVs applicable to the findings potentially relevant for classification are summarised in Table 67 of the CLH report.

In summary, the findings observed in the repeated dose toxicity studies in rats, mice or dogs either did not justify classification (consistent with CLP, Annex I, 3.9.2.8.1) or were observed at doses outside the (extrapolated) GV for classification for STOT RE 2 Therefore, RAC concludes that pyriproxyfen **warrants no classification for specific target organ toxicity-repeated exposure.**

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Pyriproxyfen was evaluated for possible genotoxic effects in four *in vitro* mutagenicity/genotoxicity assays using bacterial and mammalian cells (CLH report, table 46) and in one *in vivo* mutagenicity/genotoxicity assay in mice (CLH report, table 47).

The bacterial reverse mutation assay (Ames test) in different bacterial strains did not show any mutagenic potential of pyriproxyfen up to the highest requested dose in the absence and presence of a mammalian metabolic activation system. This result was supported by a negative outcome of the DNA-repair test using bacterial strains (Rec-assay). Pyriproxyfen did not exhibit any mutagenic potential in a gene mutation assay in mammalian cells (mouse lymphoma assay). 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, the result of the *in vivo* mouse micronucleus test was negative.

The DS concluded that considering the weight of evidence from *in vitro* and *in vivo* tests, pyriproxyfen is not genotoxic and does not require classification for germ cell mutagenicity.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

All the studies in vitro and in vivo performed on pyriproxyfen were negative.

In an adequate set of in vitro and in vivo mutagenicity/ genotoxicity assays, there was no evidence to support classification for germ cell mutagenicity.

Pyriproxyfen was negative in all tested assays in vitro and in vivo, therefore RAC concludes that no classification for germ cell mutagenicity is warranted.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS provided results of carcinogenicity studies in rats and mice exposed to pyriproxyfen in the diet (CLH report, table 49).

In the 104-week (24 months) combined chronic toxicity/carcinogenicity rat study (Vol. 3 B.6.5.1 - CA 5.5.1/01(1991a, 1994)), a decrease of the body weight gain was observed at 600 and 3000 ppm (as well as a reduction in food consumption).

Liver clinical parameters indicated increased serum cholesterol level, γ-glutamyl transferase and alkaline phosphatase only 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. At 600 ppm, corresponding to 27.3 /35 mg/kg bw/day (M/F), there were no clinical or histopathological changes. At 3000 ppm, corresponding to 138/183 mg/kg bw/day (M/F), some clinical changes and histopathological treatment-related liver necrosis in males and females were noted.

In the combined chronic toxicity/carcinogenicity study for 78-weeks, in mice (CA 5.5.1/02 (1991b, 1994) - OECD TG451 (1981), male/female Crl: CD-1 (ICR) BR, 60/sex/dose were administered pyriproxyfen in diet at 0, 120, 600, 3000 ppm, equivalent to 0/0, 16.4/21.1, 81.3/107.3, 422.5/532.8 mg/kg bw/day, respectively.

The main observation was the reduction in survival rate of the rats at all dose levels and that the deaths were observed mostly in the second part of the study. Survival was 62, 90, 58 and 54% for males and 48, 50, 51 and 72% for females for the control and 120, 600 and 3000 ppm groups,

respectively. Survival rates showed no apparent dose-related effects. No clinical signs that could be attributed to treatment were observed.

The mean body weight at the highest dose level was generally lower than control values in males and females, in weeks 13, 26, 50 (<10%) and in weeks 13, 26, 50 and 78 (12 to -14%), respectively. In addition, growth rates for high dose males and high- and mid-dose females were significantly lower than the control values.

Mean food consumption values for high dose rats was generally lower than controls up to week 70, at which time the food consumption for the female control group started to decline at a faster rate than the female treated groups. Significant decreases were noted for the 3000 ppm males at week 26 and females at weeks 13, 26 and 50, respectively. The mean water consumption values revealed significantly lower values in females in all treated groups at week 13.

At the highest dose level a slightly increased incidence in reduced motor activity and hunched posture in males and females were observed, the mean absolute body weights were slightly reduced throughout the study period for males, a statistically significant decrease in haemoglobin in females and in value of MCV in males. After the interim sacrifice effects in organs were also observed at 600 ppm and 3000 ppm. The absolute liver weights were increased at both dose levels, and relative liver weights were increased at 3000 ppm in females.

The results of the final necropsy indicated a slight increase in the relative liver weights at 3000 ppm in males and females and for males starting at 600 ppm.

Histopathological examination of the kidney presented a decrease of the absolute weights in males at the dose level of 3000 ppm in the end of the study. 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 and testes.

A severe increase in amyloidosis was noted at 600 and 3000 ppm in males and at 3000 ppm in females.

Histopathological examination of the kidneys at 3000 ppm revealed mineralisation of the renal tubules in females following unscheduled deaths and at terminal sacrifice, chronic progressive nephropathy in males and in females at interim and terminal sacrifice and segmental cortical atrophy in females at interim and terminal sacrifice.

Considering the reduced survival rate at all dose levels, the lowest adverse effect level was considered to be 120 ppm.

In this study, at 600 ppm carcinogenicity of pyriproxyfen was potentially supported by a slightly increased incidence of liver haemangiosarcoma in females (0, 1, 1, 3 for control and increasing with dose up to 5% at the HD), compared to the HCD range of 0 – 4.0% with a mean value of 0.8%. No effect was observed in males.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The high incidence of haemangiosarcoma in female rats, mostly at the highest dose level was associated with a low rate of survival. The DS considers the dose level for carcinogenicity (the critical effect) in this study to be >3000 ppm (422.5 /532.8 mg/kg bw/day in males and females, respectively). The low survival at the top dose exceeded what is acceptable according to OECD

Guidance Document (GD) 116 (and borderline acceptable at the mid dose). The female survival was slightly higher (acceptable, but still low).

The highest dose level of 3000 ppm resulted in the most severe effects, such as dark areas in the liver in females, liver necrosis in males, increasing the liver weights in both sexes, and a decrease in the body weight gain during the treatment period, starting at 600 ppm to 3000 ppm. Changes in clinical biochemistry were noted from 600 ppm.

Regarding the study in mice, the incidence of haemangiosarcoma in liver was observed in females 3/60, 5%) at 3000 ppm, being only slightly above the HCD of 0 – 4.0%. 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 was consistent with the concurrent control. In all cases, this lesion was associated with older animals, with the lesion observed from week 53 onwards.

No pre-neoplastic lesions or benign tumours in females were noted. 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.

Regarding the liver haemangiosarcoma in female mice, this is not enough to support a classification as Carcinogen Cat. 2, based on no statistical significance, no pre-neoplastic lesions, a marginally increase incidence, and the increase being restricted to a single sex.

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

RAC concludes that no classification of pyriproxyfen for carcinogenicity is warranted.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Two rodent generational studies were available in the CLH report (CLH report, table 52). Both studies are considered acceptable and reliable for the assessment in view of finding the weight of evidence to classify pyriproxyfen as a reprotoxic substance. Human data are not available.

In a two-generation reproduction study in rats, based on guideline OECD TG 415 (1981), (CA 56.1/01 (1991)), pyriproxyfen was administrated in the diet, to 26 Sprague Dawley Crl:CD®(SD)BR rats/sex/dose at the following concentrations: 0, 200, 1000 and 5000 ppm, equivalent to 0, 13.3, 66.7 and 333.3mg/kg bw/day for males and females. Dose levels were selected on the bases of a dose range finding study (10weeks pre-mating exposure). The study was not in compliance with the OECD TG 415 (1981), since the histopathology of liver was missing at dose levels of 200 ppm and 1000 ppm.

The results of the study showed a reduction in the body weight and food consumption 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.

The mortality rate was low and not-treatment related.

The DS noted the following results from this study:

- No changes detected between parental animals of the treated and control groups in mating indices, pregnancy rates, fertility, oestrus cycle and macroscopic observations.

- The status of the generation 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, at dose level of 1000 ppm (equivalent to 66.7 mg/kg bw/day) and above, was noted 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;
- No data were recorded to investigate pubertal attainment. No data on anogenital distance or other modifications of structure and function that are dependent on the integrity of the reproductive systems were performed.

Taking in consideration the presence of adverse effect for the system toxicity and no specific effect for the effects on sexual function and fertility at the dose levels of the study, the DS has proposed the NOAEL for reproductive toxicity of pyriproxyfen from this study as 5000 ppm, equivalent to 333.3 mg/kg bw/day, the highest dose level of the study.

DS considers this study as relevant and reliable for establishing no classification.

In a non-standard (non-guideline) combined teratogenicity/reproductive toxicity study performed in rats, (CA 5.6.1/02, and (1988a)). Pyriproxyfen was administrated by gavage to 24 Slc:SD(SPF) rats/sex/dose (Males: 12 weeks (9 weeks pre-mating), Females: 2 weeks pre-mating to GD7 at 0, 100, 300, 500 and 1000 mg/kg bw/day.

Mortality occurred in two parental females at 1000 mg/kg bw/day who died on D5 and D7 of administration of pyriproxyfen.

The DS noted the following results of this study:

- The effect level for general male toxicity was at 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 (enlarged of liver, kidney and adrenal) weights;
- No effects on development were found in this study;
- The number of *corpora lutea* and live fetuses were slightly decreased and placental weights were increased in dams at the highest dose level of 1000 mg/kg bw/day;
- The body weights of live fetuses were decreased at all exposure dose levels, with no dose-related trend;
- No morphological anomalies or variations in external, visceral and skeletal examination of foetuses were attributed to treatment;
- No treatment-related incidences in club foot (1 foetus Control group) and complications of club foot, club hand, vestigial tail and anal atresia (1 foetus at 500 mg/kg bw/day);
- No incidence in visceral anomalies were observed in 2 foetuses (2.5%) to 7 foetuses (6.3%) compared with 10 fetuses (9.5%) in the control group;
- No incidence in skeletal anomalies, but multiple vertrebral malformations in one foetus with external anomalies in the 500 mg/kg bw/day group;
- No incidence in skeletal variations compared with the control group (2.5 to 6.9% in the dosage versus 4.8%);
- The number of ossified phalanges of the forelimbs was higher in the dosage group compared to the control group and there were no decreases in the number of ossified bones suggesting retarded ossification.

Therefore, based on the results provided of these studies, the DS proposed no classification for sexual function and fertility.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Adverse effects on fertility

According to the CLP Criteria for reproductive toxicity, pyriproxyfen treatment did not result in specific findings relevant for classification. In the first study, any effects on sexual function and fertility were observed up to the highest dose level of 5000 ppm (equivalent to 333.3 mg/kg bw/day). In the non-guideline study, 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 was observed, thus not relevant for classification.

Thus, the DS concluded that pyriproxyfen does not warrant classification for reproductive toxicity/effects on fertility according to CLP since neither effects on sexual function nor on fertility were observed in a two-generation reproductive toxicity study.

On the basis, of the above RAC concludes **that no classification for reproductive toxicity/effects on fertility is warranted.**

Adverse effects on development

Six studies performed in rats (5) and one in rabbits were available in the DS report on pyriproxyfen. These are summarised below.

Method	**Species**	**Test material**	**Results**	**Reference**
Study 1 Non-guideline 24/sex/dose Acceptable	Rat (SD) Males: 12 wks (9 wks pre-mating) Females: 2 wks pre-mating to GD7	S-31183 (Pyriproxyfen) 97.2% 0, 100, 300, 500, 1000 mg/kg bw/day (gavage)	Parental (male) toxicity effect level:100 mg/kg bw/day Maternal toxicity effect level: 300mg/kgbw/day Developmental effect level >1000 mg/kgbw/day	CA 5.6.1/02, 1988a
Study 2 Non-guideline 23-24 (F)/dose Acceptable	Rat (SD) GD17-LD20	S-31183 (Pyriproxyfen) 97.2% 0, 30, 100, 300, 500 mg/kg bw/day (gavage)	Maternal toxicity effect level: 300 mg/kg bw/day Developmental toxicity effect level: 100mg/kg bw/day (ambulation effect in male)	CA 5.6.2/01, 1988b
Study 3 Comparable to OECD TG 414 (1981) Deviations: none Acceptable	Rat (SD) 36-47 (F)/dose (20-23 F sacrificed on GD 21)	S-31183 (Pyriproxyfen) 97.2% 0, 100, 300, 1000 mg/kg bw/day	Maternal toxicity effect level: 300mg/kg bw/day Developmental toxicity (critical effect) level: 100mg/kg bw/day (opening of the foramen transversarium of the 7th cervical vertebra)	CA 5.6.2/02, 1988c, 1989
Study 4 Comparable to OECD TG 414 (1981)Acceptable Low no. of dams at high dose	Rabbit (Japanese White) 12-14 (F)/dose GD 6-18	S-31183 (Pyriproxyfen) 97.2% 0, 100, 300, 1000 mg/kg bw/day	Maternal toxicity effect level: 300mg/kg bw/day Developmental toxicity (critical effect) level: 300 mg/kg bw/day	CA 5.6.2/03, 1988, 1994
Study 5 OPPTS 890.1500 (2009)	Rat (SD), peripubertal 15 males/dose	S-31183 (Pyriproxyfen)99.5 %	Effect dose level:1000 mg/kg bw/day	CA 5.8.3/01, 2012a

Acceptable	PND23-PND53/54	0, 500, 1000 mg/kg bw/day (gavage)		
Study 6 890.1450 Acceptable	Rat (SD), peripubertal 15 females/dose PND22-PND42/43	S-31183 (Pyriproxyfen)99.5 % 0, 500, 1000 mg/kg bw/day (gavage)	Effect dose level:1000 mg/kg bw/day	CA 5.8.3/02, 2012b

No human data on adverse effects on development are available.

In Study 1, some adverse effects were observed in males at 100 mg/kg bw/day through identification of the higher adrenal weights, without a correlation with the results from the histopathological examination being considered as a parental toxicity. No evidence of developmental effects were seen at any dose level.

Maternal toxicity was noted at 300 mg/kg bw/day based on the changes in clinical signs (diarrhoea), decreased body weights, increased food and water consumption, increasedf organ weights (liver, kidneys, and adrenals).

In Study 2, the second non-guideline study ((CA 5.6.2/01, (1988b)), perinatal/postnatal toxicity was noted in females, as maternal toxicity observed at 300 mg/kg bw/day, based on the mortality (3 out of 24 females), salivation (transient, 4/24)), clinical signs (soft stools and diarrhoea (22/24), decreased body weights (-9%, sign) and food consumption (-50% sign), increased water consumption (+25 to +60% sign) and significantly increased liver weights (+18%).

Histopathological examinations noted a gross pathology such as 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).

A developmental toxicity effect at 100 mg/kg bw/day was shown by a statistically significant changes in ambulation in males.

Maternal toxicity (mortality) was noted at 500 mg/kg bw/day . A significant reduction in the body weights of live newborns were observed at 300 and 500 mg/kg bw/day as well as a significant reduction of the offspring survival rates on D4 and at weaning at 500 mg/kg bw/day.

After the morphological examinations as well as functional status, sensory, learning and reproductive ability tests, no abnormalities were observed in pups.

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/day, compared to the control values.

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

The statistically significant changes in ambulation in males (CA 5.6.2/01, (1988b)) should be considered as a possible sign of developmental toxicity. The effect was not seen in females, no other effects were seen on similar endpoints, this is an unusual endpoint and it is difficult to assess the degree of adversity.

In Study 3, all the effects of maternal toxicity in study were observed at the dose level of 300 mg/kg bw/day, included mortality (1/36), clinical signs (hypoactivity 1/36; wasting 1/36; hypothermia 1/36), decreased body weights (-4% (sign) and body weight gain -20% (significant)) and food consumption, increased water consumption (+14 to +30% (sign)), macroscopic findings and associated organ weight changes, including increased relative liver weight (+26%, not significant) and relative kidney weight (+22%, not significant). No abnormalities were detected in the development of pups following functional, sensory, learning and reproductive ability tests

The dose level of 100 mg/kg bw/day is the developmental toxicity effect level based on 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

Parameters	Dose level (mg/kg bw/day)			
	0	100	300	1000
-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.

In Study 4, a developmental study performed in rabbits, the maternal toxicity was noted at 300 mg/kg bw/day, based on mortality, increased abortions and/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) and macroscopic findings.

Mortality was the reason for a limited number of live foetuses at 1000 mg/kg bw/day from a low number of dams at this high dose level. The effects at the high dose of 1000 mg/kg bw/day could not be assessed.

Multiple visceral malformations in 1 animal and a single visceral malformation in 2 animals was observed at 300 mg/kg bw/day.

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 ($p \leq 0.05$).

In Study 5, a pubertal development assay performed in intact juvenile/peripubertal male rats at concentrations of 0, 500 or 1000 mg/kg bw/day, pyriproxyfen was administrated by gavage from postnatal day 23 to postnatal day 53/54.

At the high dose of 1000 mg/kg bw/day, lower mean body weight gains (-9%, significant) 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.

The systemic toxicity was demonstrated in this study by decreased body weight and body weight gain at 1000 mg/kg bw/day, increased liver weight and hepatocellular hypertrophy at the dose levels of 500 and 1000 mg/kg bw/day, increased kidney weight and kidney tubular degeneration and dilatation at the highest dose level of 1000 mg/kg bw/day.

Some specific results for the systemic toxicity findings are provided below:

- At 500 mg/kg bw/day, increased liver weight (+14% absolute; +15% relative) and hepatocellular hypertrophy (13/15 males);
- 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);

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 $44.5^{NS,*}$ $44.5^{NS,*}$

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
* $p \leq 0.05$

In Study 6, a pubertal developmental assay was performed in intact juvenile/peripubertal female rats given pyriproxyfen at the dose levels of 0, 500 or 1000 mg/kg bw/day by gavage from postnatal day 22 to postnatal day 42/43.

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 with regular oestrus cycles.

Systemic toxicity was observed in the results of the studies. This included decreased body weight and body weight gain at the highest dose level of 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. At the high dose level of 1000 mg/kg bw/day a lower mean body weight gain (-7%, sign) was 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			

Parameter	♀ (mg/kg bw/d)		
	0	500	1000
- 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

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

Discussion of effects and conclusion on classification

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.

The rat developmental study (CA 5.6.2/02, 1988c) presented a statistically significant increase in the proportion of foetuses, with a skeletal variations at the highest dose level of 1000 mg/kg bw/day.

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 (7th 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

At the highest dose level, the main findings are related to:

- A significant increase in the incidence of a single variation; namely opening of *foramen transversarium* of the 7th cervical vertebra; the incidence 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.

A developmental LOAEL was set at 100 mg/kg bw/day during the pesticide peer review meeting (PPR 190, Jan-Feb 2019). This finding was associated with the maternal toxicity in all dose groups, associated with the high level of mortality at the top dose of 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 not relevant 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) reported 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 of 100 mg/kg bw/day is clearly within the historical control range. The incidences at 300 and 1000 mg/kg bw/day exceeded the historical control range, but were associated with maternal toxicity.
- Compared with the HCD, the increased incidence of the specific finding (opening of the 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.
- The other foetal skeletal parameters were unaffected by treatment with pyriproxyfen.

The standard developmental rabbit study (CA 5.6.2/03, 1988) noted the following findings:

- A higher incidence of abortion 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 GD 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 GD 9-22, and was lower (although not significantly) at 300 mg/kg bw/day.
- The high incidences of mortality and abortion, associated with the limited number of litters available for assessment at the highest dose level of 1000 mg/kg bw/day.
- 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*

At the highest dose level of 1000 mg/kg bw/day, the assessment was based on a low number of litters. There was an insufficient number of animals for a comprehensive foetal evaluation.

At 300 mg/kg bw/day the incidence of foetal visceral malformations was greater than in controls, this finding is largely attributable to a single foetus with multiple malformations.

Statistically, 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 during 1982-1988); one study in the historical control range reports three foetuses (3.3%) with visceral malformations. The dose level of 1000 mg/kg bw/day was not appropriate for this study, thus all malformations had a single incidence, did not attain statistical significance and as appeared to be a spontaneous reaction at the high level of toxicity.

The low number of litters available for assessment at the high dose level is normally compared with the complete absence of foetal visceral malformations. During the pesticide peer review meeting (PPR 190, Jan-Feb 2019), it was concluded to set the developmental NOAEL at 100 mg/kg bw/day based on multiple visceral malformations in one animal and single visceral malformations in two animals at 300 mg/kg bw/day and the fact that the high dose level of 1000 mg/kg bw/day could not be assessed due to an insufficient number of dams remaining in experimental study. However, considering the incidence of visceral malformations within the historical control data and the reported malformations found with a single incidence, no classification for developmental toxicity is warranted.

Comparing with the CLP criteria for reproductive effect and developmental toxicity, the first study, a non-standard rat study in which females were exposed to pyriproxyfen by gavage for two weeks pre-mating and up to GD 7 reported 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 classification is not warranted.

In the second study, a non-standard study (CA 5.6.2/01, 1988b), the findings were compared with the criteria of CLP regulation in view of the developmental classification and/or reproductive toxicity classification: 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 that 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.

The pubertal development study in male rats which 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) mentioned a lower body weight and body weight gain at the high dose level of 1000 mg/kg bw/day.

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 in favour of classification as developmental toxicity.

In the pubertal development study performed in female rats 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) were noted a lower mean body weight gain and a delay in the mean age of attainment of vaginal opening 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 in favour of classification as developmental toxicity.

Therefore, the dose level of 1000 mg/kg bw/day was inappropriate for assessing the developmental toxicity of pyriproxyfen. The systemic toxicity was evident and the high mortality demonstrated at the highest dose level did not allow the application of the criteria of CLP Regulation.

RAC concurs with the opinion of DS that **no classification for effects on development is warranted**.

Effects on or via lactation

No additional study was carried out.

Comparison with the CLP criteria

Adverse effects on or via lactation are included in the assessment of reproductive toxicity, but for classification purposes such effects are treated separately. 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 dams. Based on the criteria for effects on or via lactation, the two-generational study in rats, according to the OECD TG 416 (1981), CA 5.6.1/01 (1991)

are relevant. The results of the study revealed a decrease of the pup body weight development in F1 and F2 pups during lactation at 5000 ppm (333.3 mg/kg bw/day) (the highest dose level).

In the F1 generation the pup body weight reduction was significantly greater than in the F2 generation.

- Reduced F1 pup body weight development (-16%, significant)
- Reduced F2 pup body weight development (-13%, significant)

The effect was observed during the last week of lactation, when pups already started to be fed. Therefore, the pups were exposed via the diet during the last stage of the study.

The maternal toxicity was observed at 5000 ppm and was expressed in the reduced pup body weights during the last week of lactation.

RAC concluded that no classification for effects on or via lactation is warranted.

Conclusion on classification and labelling for reproductive toxicity

RAC agrees with the conclusion of the DS regarding the classification of pyriproxyfen for effects on reproduction and development. Pyriproxyfen does not meet the CLP classification criteria for toxicity to reproduction.

Overall conclusion on reproductive toxicity

RAC concludes that **no classification is warranted for effects on sexual function and fertility, developmental toxicity or effects on or via lactation**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Pyriproxyfen is classified as Aquatic Acute 1 and Aquatic Chronic 1 in Annex VI of Regulation (EC) No 1272/2008 (CLP). The DS proposed to retain these and add an M-factor of 10 to the Acute 1 classification based on a 96-hour EC_{50} of 0.065 mg/L for *Mysidopsis bahia* ($0.01<L(E)C_{50} \leq 0.1$ mg/L) and to add an M-factor of 10 000 to the Chronic 1 classification based on a 21-day EC_{10} of 0.0000088 mg/L for *Daphnia magna* ($0.000001<EC_{10}\leq 0.00001$ mg/L for a not rapidly degradable substance).

Degradation

The DS concluded that pyriproxyfen is not readily biodegradable. In a closed bottle ready biodegradability test comparable to OECD TG 301C (CA 7.2.2.1/01, RAR B.8.2.2.1, GLP), pyriproxyfen degraded 0.69% in 28 days. The test solution was stirred continuously. The study design did not include a toxicity control. However, in an activated sludge respiration inhibition test (OECD TG 209, GLP), only 2% inhibition was observed at the highest tested concentration of 100 mg/L.

The DS concluded pyriproxyfen to be hydrolytically stable at pH 4, 7 and 9. Hydrolysis as a function of pH study (OECD TG 111, GLP) was conducted with [phenyl-^{14}C] and [pyridyl-^{14}C]-pyriproxyfen at pH 4, 7 and 9 at 50°C for 7 days (CA 7.2.1.1/01, RAR B.8.2.1.1). Degradation half-lives were greater than 367 days in all buffer solutions.

Aerobic mineralisation of [^{14}C]-pyriproxyfen in surface water was investigated in a GLP OECD TG 309 study (CA 7.2.2.2/01, RAR B.8.2.2.2). The radiolabelled test item, separately labelled in two positions (pyridyl and phenyl) was applied to natural pond water at nominal test item concentrations of 0.05 and 0.005 mg/L. 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 and 5.0 days for the low dose test systems. These degradation half-live values correspond to 30.8 and 10.6 days, respectively, when normalised to 12 °C.

Lewis (CA 7.2.2.3/01, RAR B.8.2.2.3, GLP) 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). The data from this study were analysed according to FOCUS guidance (2006, 2011) (CA 7.2.2.3/03). The test substance was separately radiolabelled in two positions (pyridyl and phenyl). The pseudo-SFO DT_{50} values of 22.2 days for Mill stream pond and 27.8 days for Emperor Lake at 20 °C were derived. Normalization to 12 °C yielded degradation DT_{50} values of 47.1 and 59.0 days for the total systems, respectively. Mineralisation after 100 days was from 11 to 53%. Non-extractable residues after 100 days were from 31 to 51%.

Based on the above the DS considered pyriproxyfen as not rapidly degradable.

An aqueous photolysis study of [pyridyl-^{14}C]pyriproxyfen was conducted to determine the photolysis quantum yield (CA 7.2.1.2/03, GLP). 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. The half-life of pyriproxyfen in light exposed samples was determined as 10.5 hours of continuous irradiation.

Bioaccumulation

There were two fish bioaccumulation studies available. Bioaccumulation of ^{14}C-labelled pyriproxyfen was studied in flow-through conditions according to US EPA 165-4 (CA 8.2.2.3/01a, b, c; RAR B.9.2.8.2, GLP). *Lepomis macrochirus* were exposed to 0.02 mg/L pyriproxyfen for 28 days. The reported steady-state BCF values based on total radioactivity were 1379 L/kg for phenyl-^{14}C, and 1495 L/kg for pyridyl-^{14}C for whole body. Lipid content was not determined in the study and the BCF values have not been normalised to 5% lipid. Also, bioconcentration was determined only at one concentration, not allowing to conclude the possible dependency between BCF and exposure concentration. The DS considered the BCF values an underestimate and used all uptake and depuration phase data available to calculate kinetic BCF values yielding whole fish BCF values of 1489 and 1653 L/kg for the phenyl-^{14}C and pyridyl-^{14}C labelled pyriproxyfen treatments, respectively.

A supportive bioaccumulation study on *Cyprinus carpio* according to EHWD No.5, PAB No.615, BIB No.392 (CA 8.2.2.3/02a, RAR B.9.2.8.2, GLP) was also presented by the DS. Carps were exposed to 0.5 and 5.0 µg/L pyriproxyfen for 8 weeks. BCF values of 290-850 and 230-900 for the low and high treatment, respectively, were reported. The study was not considered acceptable in the RAR due to several limitations, but the DS considered the study to support that pyriproxyfen has a potential for bioaccumulation.

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 (RAR B.2.7).

In conclusion, the DS considered pyriproxyfen to have a potential for bioaccumulation.

Acute Aquatic Toxicity

Table: *Reliable acute aquatic toxicity data on pyriproxyfen*

Method	Species	Test material	Results	Reference
Fish				
US EPA 72-1; GLP flow-through **	*Oncorhynchus mykiss*	Technical pyriproxyfen 95.3%	96h-LC_{50}: >0.325 mg/L (mm) start: mm 74 ± 11% of nominal end: mm 74 ± 26% of nominal	CA 8.2.1/01; RAR B.9.2.1.1
US EPA 72-1; GLP flow-through ***	*Lepomis macrochirus*	Technical pyriproxyfen 95.3%	96h-LC_{50}: >0.270 mg/L (mm) start: mm 81.4 ± 8.2% of nominal end: mm 71.6 ± 4.5% of nominal	CA 8.2.1/02; RAR B.9.2.1.2
Aquatic invertebrates				
US EPA 72-2; GLP flow-through ***	*Daphnia magna*	Technical pyriproxyfen 95.3%	48h-EC_{50}: 0.40 mg/L (mm) start: mm 80 ± 8.9% of nominal end: mm 66 ± 16% of nominal	CA 8.2.4.1/01; RAR B.9.2.4.1
US EPA 72-3; OPPTS 850.1025; GLP flow-through ***	Eastern oyster (*Crassostrea virginica*)	Technical pyriproxyfen 97.1%	96h-EC_{50}: 0.092 mg/L (mm) start: mm 63-89% of nominal end: mm 50-79% of nominal	CA 8.2.4.2/01; RAR B.9.2.4.6
OPPTS 850.1035; GLP flow-through ***	*Mysidopsis bahia*	Technical pyriproxyfen 97.1%	**96h-EC_{50}: 0.065 mg/L (mm)** start: mm 62 -75% of nominal end: mm 70-105% of nominal	CA 8.2.4.2/02; RAR B.9.2.4.7
Algae or other aquatic plants				
OECD 201; GLP static ***	*Raphidocelis subcapitata*	Technical pyriproxyfen, 97.2%	72h-E_rC_{50}: 0.111 mg/L (mm) mm 69-83% of nominal	CA 8.2.6.1/01; RAR B.9.2.6.1
US EPA 122-2 and 123-2; GLP semi-static ***	*Lemna gibba*	Technical pyriproxyfen 98.4%	14d-$EC_{50,\ frond\ density}$: >0.18 mg/L 14d-$EC_{50,\ biomass}$: >0.18 mg/L no effects at the highest treatment	CA 8.2.7/01; RAR B.9.2.7.1

			*at day 6, new: 61-102% of nominal at day 9, old: 24-31% of nominal	

mm= mean measured
* 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.
** solvent used (not specified)
*** acetone used as solvent
In addition to the studies presented in the Table above and considered reliable, the DS considered an acute toxicity study with *Cyprinus carpio* (US EPA 72-3; GLP; CA 8.2.1/05; RAR B.9.2.1.5) acceptable with restrictions. The 96h-LC_{50} was >0.328 mg/L which was the maximal solubility in the test medium. Mean measured concentrations were 20-35% of nominal indicating that the test was conducted above the solubility limit in seawater. Insoluble material was observed throughout the test at all concentrations.

There were reliable data available on fish, invertebrates, algae and aquatic plants. The lowest acute toxicity value was a 96h-EC_{50} of 0.065 mg/L for *Mysidopsis bahia*.

Chronic Aquatic Toxicity

Table: *Reliable chronic aquatic toxicity data on pyriproxyfen*

Method	Species	Test material	Results	Reference
Fish				
US EPA 72-4; GLP, FELS-test flow-through *	*Oncorhynchus mykiss*	Technical pyriproxyfen 97.2%	95d- NOEC (length, wet weight): 0.0043 mg/L (mm) (1 mm 87-113% of nominal	CA 8.2.2.1/01; RAR B.9.2.2.2 CA 8.2.2.1/01b; RAR B.9.2.2.3
FFLC TG, Japanese ministry of the environment (Annex 6-2); GLP flow-through ***	*Oryzias latipes*	Technical pyriproxyfen 98.7%	189d-NOEC (based on overall hatchability of F1 generation): 0.0027 mg/L (mm) (2 mm 84-86% of nominal	CA 8.2.2.2/01; RAR B.9.2.2.4
Invertebrates				
US EPA 72-4; GLP flow-through, *	***Daphnia magna***	**[pyridyl-2,6-^{14}C]-pyriproxyfen, Radiochemical purity: 100%**	**1st test: 21d NOEC: 0.000015 mg /L (mm) 2nd test 21-day NOEC <0.000020 2nd test: 21d-EC_{10} : 0.0000088 mg/L (mm) mm 75-111% of nominal**	**Blakemore *et al*. (1992) CA 8.2.5.1/01 Lewis (2016); CA 8.2.5.1/01b; RAR B.8.2.5.1**
US EPA 72-4; ASTM E1191 –	*Mysidopsis bahia*	Technical pyriproxyfen	28d-NOEC (reproduction):	CA 8.2.5.2/06 RAR B.9.2.5.11

87; GLP flow-through **		95.3%	0.00081 mg/L (mm) (3 mm 62-77% of nominal	
Algae and aquatic plants				
OECD 201; GLP static **	*Raphidocelis subcapitata*	Technical pyriproxyfen 97.2%	72h-NOEC: 0.02 mg/L (mm) (2 mm 69-83% of nominal	CA 8.2.6.1/01; RAR B.9.2.6.1
US EPA 122-2 and 123-2; GLP semi-static **	*Lemna gibba*	Technical pyriproxyfen 98.4%	14d-NOEC >0.18 mg/L (mm) mean measured: *at day 6, new: 61-102% of nominal at day 9, old: 24-31% of nominal	CA 8.2.7/01; RAR B.9.2.7.1

mm=mean measured
(1 EC_{10} calculated by Anonymous 2016 but considered not reliable due to the bad fit.
(2 EC_{10} value could not be calculated
(3 EC_{10} calculated but considered unreliable
*DMF used as solvent ** acetone used as solvent *** solvent used (not specified)

There were reliable data available on fish, invertebrates, algae and aquatic plants. The DS considered a 21d-EC_{10} of 0.0000088 mg/L for *Daphnia magna* to be the lowest chronic test result. The study was performed by Blakemore *et al*. (1992) and the data were reanalysed by Lewis (2016). Two definitive tests were reported both consisting of a control, solvent control 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. Analytical measurements were conducted in composite samples taken from the four replicates at days 0, 4, 7, 14 and 21. 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. There were no significant differences between control and solvent control in either test with respect to the investigated endpoints. Treatments were therefore compared to the pooled controls in both tests.

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 ($P \leq 0.05$) from the solvent control.

**: Denotes values significant different ($P \leq 0.05$) from the pooled controls.

In the 1st test, no significant effects were observed for any of the treatments compared to the pooled control even at the highest test concentration of 31 ng/L. In the 2nd test, 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 and a 21d-NOEC of 20 ng/L.

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). The RAR concluded that the slight but significant effects observed in the second study are not due to the use of the solvent, 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 by the DS. The DS agreed with the comment in the RAR that slight differences in outcome of similar test are not uncommon, even when performed under highly similar conditions. 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 DS fitted a log-logistic model to the available reproduction summary data from the 2nd test and obtained a slightly higher 21d-EC_{10} of 12.3 ng/L. Considering, that the applicant had the availability of the raw data and that both values are close to each other, the DS concluded that the 21d-EC_{10} of 8.8 ng/L can be used for classification purposes.

The DS also presented an Amphibian metamorphosis assay (US OPPTS 890.1100, OECD TG 231; GLP; CA 8.1.4/01; RAR B.9.1.4.1) with *Xenopus laevis* giving a 21d-NOEC (reduced hindlimb length) 0.0017 mg/L (mm). Although considered reliable, RAC is of the opinion that the endpoint is not relevant for aquatic hazard classification.

The DS also presented a freshwater invertebrate chronic toxicity sediment-water test with *Hyalella azteca* (US EPA OCSPP 850.1770; GLP; CA 8.2.5.4/01; NNW-0243; RAR B.9.2.5.14). Pyriproxyfen was applied to sediment. During the test 85-99% of pyriproxyfen remained associated with sediment/pore water. 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. Although considered reliable, RAC is of the opinion that the test is not relevant for aquatic hazard classification due to possible exposure via sediment.

Also, a reliable sediment-water chironomid toxicity test (spiked water) test was presented by the DS but considered not relevant for aquatic hazard classification by RAC. The 28d-NOEC for *Chironomus riparius* based on emergence was 0.031 mg/L (overlying water). The concentration in overlying water was 17-27% of nominal from day 7 onwards and the concentration of pyriproxyfen showed corresponding increases in the pore water and in the sediment. RAC is of the opinion that sediment exposure cannot be ruled out.

Comments received during consultation

Two Member States supported the proposed classification. A National Authority (NA) commented on the *Daphnia magna* reproduction study by Blakemore *et al.* (1992) and Lewis (2016). They asked for the solvent concentrations in controls and treatments for Test 1 and Test 2 to find out if solvent controls in treatments have exceeded the solvent control concentration. The DS answered that, for reproduction, the solvent control was not statistically different from the blank

control. Thus, the controls were correctly pooled for the statistical analysis and conclusion by DS. Furthermore, 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.

The NA also commented on the 21-day NOEC of 15 ng/L from Test 1. They were unclear of the basis of this endpoint given no statistically significant effects were observed in Test 1 when treatments were compared to pooled or solvent controls. They believed a statistically significant 21-day NOEC ≥31 ng/L should take precedence.

They also noted that the quoted 21-day EC_{10}(reproduction) of 8.8 ng/L is derived from effects observed at all treatments in Test 2. It is below the lowest treatment (20 ng/L, mm) and therefore outside of the model. OECD and ECHA guidance (ECHA, 2010) recognise that estimated EC_x values outside the concentration-response modelling are subject to a great deal of uncertainty. In addition, the 95% CIs of 2.6 to 16 ng/L span 2 hazard classification bands. Due to the uncertainty, they considered that a NOEC may be more statistically reliable in this instance. Alternatively, they noted a 21-day EC_{20}(reproduction) of 18 ng/L was also available which was just below the lowest Test 2 treatment of 20 ng/L which represents the Test 2 NOEC if comparing to the solvent control. Considering the Test 2 EC_{10}(reproduction) of 12.3 ng/L calculated by the DS, the Test 1 NOEC, EC_{20} and potential Test 2 NOEC, they believed a weight of evidence assessment supported a chronic M-factor of 1000.

RAC took note of the comments made by the NA regarding the EC_{10} value.

A MS pointed out that there are mesocosm studies (R.P.A van Wijngaarden, 2004) with a $LOEC_{community}$ of 5 µg a.s./L. They asked if an EC_{50}, which might change the acute M-factor, is available for this study. They also informed that the BPR dossier contains an efficacy test on *Aedes aegypti* from which EC_{50} (6h) of 21.4 ng/L and wondered if this endpoint should be considered for the acute classification.

RAC does not consider the plankton dominated microcosm experiment with the formulation Pyriproxyfen EC10 relevant or reliable for aquatic hazard classification purposes. There is no EC_{50} available in the study report. The CLH Report states: "*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*."

The guideline for the *Aedes aegypti* study was WHO (1981) Instruction for determining the susceptibility or resistance of mosquito larvae to insect developmental inhibitors (WHO/UBC/91.812). The result mentioned is from a continuous exposure method (the larvae were exposed continuously to the test solution until emergence). Test concentrations were not measured, and RAC is of the opinion that the results are not reliable for classification purposes. (Document IIIA Section 7 of the CAR, May 2012).

Assessment and comparison with the classification criteria

Degradation

RAC concludes that pyriproxyfen is not rapidly degradable, as proposed by the DS, based on:

- 0.69% degradation after 28 days in an OECD TG 301C test was lower than the pass level 60% in the CLP Criteria

- degradation DT_{50} values of 14.5 and 5.9 days at 20 °C and 30.8 and 10.6 days at 12 °C (OECD TG 309) – no ultimate degradation in the aquatic simulation test
- pyriproxyfen was hydrolytically stable at pH 4, 7 and 9 (OECD TG 111)
- DT_{50} values of 22.2 and 27.8 days at 20 °C and 47.1 and 59 days at 12 °C in a water/sediment study – pass level of the CLP Criteria DT_{50} <16 days was not fulfilled.

Bioaccumulation

RAC concludes that pyriproxyfen has a potential for bioaccumulation, as proposed by the DS, based on:

- Fish BCF values of 1379 and 1495 L/kg are greater than the cut-off value of 500 of the CLP Criteria
- log K_{ow} values 4.85 at pH 5, 4.86 at pH 7, and 4.87 at pH 9 are greater than the cut-off of 4 of the CLP Criteria.

Acute Aquatic Toxicity

RAC concludes that the lowest acute toxicity value was a 96h-EC_{50} of 0.065 mg/L for *Mysidopsis bahia* (CLP Annex I: Table 4.1.0 (a)). The EC_{50} is in the $0.01<LC_{50}\leq0.1$ mg/L range, **warranting classification as Aquatic Acute 1, with an M-factor of 10.**

Chronic Aquatic Toxicity

Additional information was submitted to the RAC after the consultation in relation to the re-analysis made by the DS confirming the chronic M-factor of 1000.

In the re-analysis of the *Daphnia magna* study, the DS concluded that Lewis (2016) performed its analysis on the combined dataset derived from the 1st and 2nd study and, therefore, the EC_{10} of 8.8 ng/L for reproduction is not considered acceptable for classification purposes. They noted that while no significant effects were observed for any of the analysed endpoints compared to the pooled control in Test 1, the applicant observed for adult length and YAD (young per adult per reproductive day) a slight trend towards decreased length and lower YAD at the 31 ng/L level and concluded a NOEC of 15 ng/L. Regarding Test 2, the DS calculated in accordance with OECD TG 211 (version 2012) an **EC_{10} of 13.3 ng/L** (95% Ci of 8.45 to 18.30) for reproduction, based on the total number of living offspring per parent animal (which equals to the production of living offspring by the surviving parent organisms as there were no mortalities for any of the treatments). The DS considered this EC_{10} reliable without restrictions, warranting a classification as Aquatic Chronic 1, M-factor of 1000.

The DS also submitted to RAC a report received from the applicant regarding the calculation of EC_{10} and EC_{20} values from the Blakemore *et al*. (1992) *Daphnia magna* study. It was noted that the reproductive output in this study was only expressed as young (offspring)/adult reproductive day, which is not the preferred effects endpoint as reported in the test protocol. A re-evaluation was performed, and the required parameters were calculated. *i.e.* total offspring and number of offspring/surviving adults. The report concluded that due to the poor confidence intervals and other statistical parameters, the estimated/calculated EC_{10} values both the original ones from Lewis (2016) and the newly estimated ones (ranging from 10.57 to 16.51 ng/L) should be considered with caution. These newly estimated values concurred with the NOEC value derived in the study report of 15 ng/L. It was concluded that the overall chronic endpoint from this study can be considered to be the NOEC of 15 ng/L.

RAC agrees with the DS's revised opinion and reasoning not to consider the EC_{10} of 8.8 ng/L acceptable for classification purposes and to base, instead, the chronic classification on the EC_{10} of 13.3 ng/L from Test 2, supported by the NOEC of 15 ng/L.

In conclusion, RAC concludes, after the re-assessment of the *Daphnia magna* study results, to

consider the Test 2 21d-EC_{10} of 13.30 ng/L as the basis for chronic classification (CLP Annex I: Table 4.1.0 (b) (i)). The NOEC of 15 ng/L from Test 1 and the combined study (Test 1+Test 2) is in the same classification range. The EC_{10} is in the $0.00001<EC_{10}\leq0.0001$ mg/L range, warranting an M-factor of 1000. Overall, RAC concludes that a classification as **Aquatic Acute 1, M=10** and **Aquatic Chronic 1, M=1 000** is warranted.

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

Pyriproxyfen is non-volatile based on the vapour pressure of $<1.33 \times 10^{-5}$ Pa at 22.81°C and on the Henry's law constant of $<7.37x10^{-2}$ $Pa.m^3/mol$ (23°C).

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). The DT_{50} 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 guidance methodology to determine the potential of a substance for atmospheric ozone depletion considers the following issues relevant:

1. The atmospheric lifetime 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);

Pyriproxyfen is not volatile, has a short atmospheric residence time, does not contain F, Cl or Br and is, consequently, not hazardous to the ozone layer.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC concludes **that the criteria for classification as hazardous to the ozone layer are not met**. Its properties and its predicted or observed environmental fate and behaviour does not indicate that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer. It is not volatile, has a short atmospheric residence time, and does not contain F, Cl or Br.

There is no information of the Ozone Depleting Potential of pyriproxyfen to compare if it is greater or equal to the lowest ODP of the substances currently listed in Annex I to Regulation (EC) No 1005/200989.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).