Committee for Risk Assessment

RAC

Annex 1

Background document

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

pyroxsulam (ISO); *N*-(5,7-dimethoxy[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide

EC Number: -

CAS Number: 422556-08-9

CLH-O-0000001412-86-102/F

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

Adopted

10 March 2016

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Pyroxsulam

EC Number: Not assigned

CAS Number: 422556-08-9

Index Number: Not assigned

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Version number: **2** **Date:** **May 2015**

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SCIENTIFIC EVALUATION OF THE DATA

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Pyroxsulam***
EC number:	Not assigned
CAS number:	422556-08-9
Annex VI Index number:	None available
Degree of purity:	≥ 96.5 %
Impurities:	Confidential – not relevant to the CLH proposal

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	None
Current proposal for consideration by RAC	Skin Sens 1; H317 – May cause an allergic skin reaction Aquatic Acute 1; H400 - Very toxic to aquatic life (Acute M factor = 100) Aquatic Chronic 1; H410 - Very toxic to aquatic life with long lasting effects (Chronic M factor = 100)
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Skin Sens 1; H317 – May cause an allergic skin reaction Aquatic Acute 1; H400 - Very toxic to aquatic life (Acute M factor = 100) Aquatic Chronic 1; H410 - Very toxic to aquatic

	life with long lasting effects (Chronic M factor = 100)

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification

2.15.	Organic peroxides	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
	Acute toxicity - dermal	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
	Acute toxicity - inhalation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	Data lacking
3.4.	Skin sensitisation	**Skin Sens 1; H317 – May cause an allergic skin reaction**	**None**	**None**	
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification

4.1.	Hazardous to the aquatic environment	**Aquatic Acute 1; H400 - Very toxic to aquatic life** **Aquatic Chronic 1; H410 - Very toxic to aquatic life with long lasting effects**	Acute M factor = 100 Chronic M factor = 100)		
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Pictogram(s): GHS07, GHS09

Signal word: Warning

Hazard statements: H317: May cause an allergic skin reaction
H410 - Very toxic to aquatic life with long lasting effects

Precautionary statements: Not required, P statements are not included in Annex VI

Proposed notes assigned to an entry: None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Pyroxsulam is a pesticide active substance approved under Directive 91/414/EEC (subsequently replaced by EU Regulation 1107/2009), for which the UK were the Rapporteur Member State (RMS). Refer to Commission Implementing Regulation (EU) No 1176/2013 of 20 November 2013. There is no entry on Annex VI of CLP and there have been no previous classification and labelling discussions for this substance. Therefore, in accordance with Article 36(2) of the CLP Regulation, pyroxsulam should now be considered for harmonised classification and labelling. As the substance does not have a current entry on Annex VI of CLP this proposal considers all physical, human health and environmental hazard classes.

At the time of submission the substance is not registered under REACH.

2.2 Short summary of the scientific justification for the CLH proposal

Pyroxsulam is a member of the triazolopyrimidine sulfonamides, a class of herbicides known to inhibit the plant enzyme acetolacate synthase (ALS). It is broadly active on annual grass and broadleaf weeds, with some activity on certain perennial weed species.

The conclusion of the EFSA peer review process (EFSA Journal 2013;11(4):3182) noted concern for skin sensitisation and carcinogenicity. However, there was no consensus between the experts regarding the latter. Classification with Aquatic Acute 1; H400 and Aquatic Acute 1; H410 were also considered appropriate.

In a standard guinea pig maximisation study, the sensitisation response was 80 % in treated animals receiving an intradermal induction of 5% pyroxsulam. Classification in Skin Sens 1: H317 – May cause an allergic skin reaction is therefore proposed. Refer to section 4.6 of this report for full details.

Large granular lymphocyte leukaemia (LGL) in Fischer 344 rats and hepatocellular adenomas and carcinomas in mice were observed. The leukaemia was not considered to be related to treatment and therefore was not considered for classification. The increased incidence of liver adenomas and carcinomas in the mouse was slightly higher than the contemporaneous and laboratory historical control in males, but the carcinomas were within the control range provided for Charles River Labs (from which the mice were sourced). In addition, these findings occurred in male mice only, which appeared to be susceptible to liver tumour formation with multiple adenomas (rather than single incidences) observed in the livers of both control and treated animals. In conclusion, it is considered that there is insufficient evidence in this study to conclude that there is a treatment-related carcinogenic effect of pyroxsulam. Refer to 4.10 of this report for full details.

Aquatic acute toxicity data on pyroxsulam are available for fish, invertebrates, algae and aquatic plants. Aquatic plants are the most acutely sensitive trophic group. The lowest $L(E)C_{50}$ value is a 7-day E_rC_{50} of 0.00388 mg/l for *Lemna minor* in the range 0.001 to ≤0.01 mg/l. On this basis pyroxsulam should be classified as Aquatic Acute 1 with an M factor of 100.

Adequate chronic toxicity data on pyroxsulam are available for fish, invertebrates, algae and aquatic plants. The lowest value is a 7-day NOE_rC for *Lemna minor* of 0.0007 mg/l. Given this is in the

range 0.0001 to ≤0.001 mg/l and the substance is considered non-rapidly degradable, pyroxsulam should be classified as Aquatic Chronic 1 with an M factor of 100.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Not currently listed.

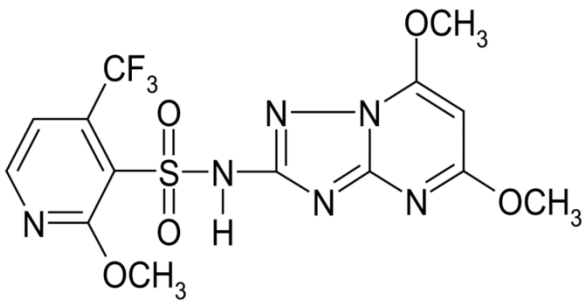
2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling

The following entries are included in the classification and labelling inventory at the time of submission

Classification		Labelling		
Hazard Class and Category Code(s)	**Hazard Statement Code(s)**	**Hazard Statement Code(s)**	**Supplementary Hazard Statement Code(s)**	**Pictograms, Signal Word Code(s)**
Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	H317 H410	-	GHS07 GHS09 Wng
Skin Sens. 1B Aquatic Chronic 1	H317 H410	H317 H410	-	GHS07 GHS09 Wng
Skin Sens. 1 Aquatic Chronic 1	H317 H410	H317 H410	-	GHS07 GHS09 Wng

RAC general comment
Pyroxsulam is a pesticide active substance approved under Directive 91/414/EEC (and subsequently replaced by EU Regulation 1107/2009). It provides broad spectrum post-emergence control of annual grasses and broadleaf weeds in winter wheat, rye and triticale, a wheat-rye hybrid.



Pyroxsulam is a triazolopyrimidine sulphonamide and is typical of this class of compounds (i.e. a substituted triazolopyrimidine connected to a substituted phenyl ring through a sulphonamide bridge). Its molecular target is inhibition of plant acetolactate synthase (ALS), an enzyme crucial to the first step in branched chain aliphatic amino acid (leucine, isoleucine and valine) biosynthesis.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Pyroxsulam is a pesticide active substance approved under Directive 91/414/EEC (subsequently replaced by EU Regulation 1107/2009), for which the UK were the Rapporteur Member State (RMS). Refer to Commission Implementing Regulation (EU) No 1176/2013 of 20 November 2013. There is no entry on Annex VI of CLP and therefore, in accordance with Article 36(2) of the CLP Regulation, pyroxsulam should now be considered for harmonised classification and labelling. All physical, human health and environmental hazard classes are considered in this report.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

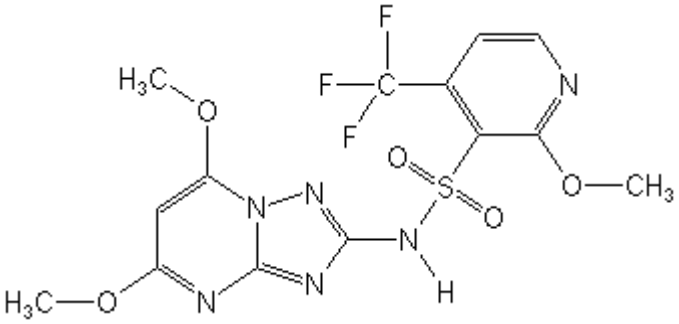
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	Not assigned
EC name:	-
CAS number (EC inventory):	-
CAS number:	422556-08-9
CAS name:	3-Pyridinesulfonamide, *N*-(5,7-dimethoxy[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-
IUPAC name:	N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide
CLP Annex VI Index number:	Not applicable
Molecular formula:	$C_{14}H_{13}F_3N_6O_5S$
Molecular weight range:	434.4

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Pyroxsulam	≥ 96.5 g/kg		

Current Annex VI entry: None

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Confidential			

There are a number of process impurities in the substance. These have been taken into consideration and are not considered to impact on the classification proposed in this dossier. Further information on the impurities is considered to be confidential but full details are provided in the technical dossier.

Current Annex VI entry: Not applicable

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

Current Annex VI entry: Not applicable

1.2.1 Composition of test material

The batches used in the relevant studies were considered to be equivalent to the manufactured material during the review of the active substance under Dir 91/414/EEC.

1.3 Physico-chemical properties

Table 8: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated etc.,)
State of the substance at 20°C and 101,3 kPa	White crystalline solid	S. Madsen 2006a, 2006c	Visual observation 99.3%
Melting/freezing point	208.3 °C	S Madsen 2006a	OPPTS 830.7200 ASTM E967-92 99.3% GLP
Boiling point	Decomposes at 213 °C (immediately after melting) before boiling.	S Madsen 2006a	EEC Method A1/A2 99.3%
Relative density	1.618	S. Madsen, R. Kastel 2003	EEC Method A3 99.3% GLP
Vapour pressure	< 1x10-7 Pa at 20 °C	S. Madsen, R. Kastel, 2003	OECD Guideline 104 (thermogravimetic method) 99.3% GLP
Surface tension	Not surface active 62.3 mN/m at 20 °C (0.01% solution) 63 mN/m at 20 °C (1.0% solution)	S. Madsen, R. Katsel, 2003	EEC Method A5 99.3% GLP
Water solubility	At 20°C 0.0626 g/l (purified water) 0.0164 g/l pH4 3.20 g/l pH7 13.7 g/l pH9	B. Turner 2004a	EEC Method A6 99.3% GLP
Partition coefficient n-octanol/water	At 20 °C LogPow = 1.08 pH 4 buffer solution LogPow = -1.01 pH7 buffer solution LogPow = -1.60 pH 9 buffer solution	B. Turner 2004b	EEC Method A8 (shake flask) 99.3% GLP
Flash point	Not applicable substance is a solid		
Flammability	Sample ignited but failed to sustain combustion for more than 2 seconds. Experience in handling and use indicates that it is not a pyrophoric solid and does not emit flammable gas on	B. Turner 2005	EEC Method A10 98% GLP

	contact with water.		
Explosive properties	Not explosive. No evidence of ignition or explosion but slight decomposition indicated.	B Turner, 2005	EEC Method A14 98% GLP
Self-ignition temperature	No self ignition < 400 oC	B Turner 2005	EEC Method A16 98% GLP
Oxidising properties	Not oxidising. Charred but did not burn to completion	B. Turner, 2005	EEC Method A17 98% GLP
Granulometry	No data		
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	At 20 ^{o}C pKa = 4.67 (deprotonation of the nitrogen at the sulphonamide location occurs at higher pH)	C. Cathie, 2004	OECD Test Guideline 112 100% GLP
Viscosity	Not applicable		

Reference should be made to the Draft Assessment Report (DAR) – Pyroxsulam - Volume 3, Annex B2: Physical and Chemical Properties – January 2012

2 MANUFACTURE AND USES

2.1 Manufacture

Pyroxsulam is manufactured outside of the EU.

2.2 Identified uses

Pyroxsulam is placed on the market within the EU as an herbicide.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 9: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Refer to table 8			

3.1 Physico-chemical Hazards

3.1.1 Summary and discussion of Physico-Chemical Hazards

3.1.2 Comparison with criteria

In a standard flammability study (EEC Method A10), pyroxsulam ignited but failed to sustain combustion. As such, it does not meet the criteria for classification as a flammable solid. The self ignition temperature was found to be > 400 ^{o}C. Further, experience in handling and use indicates that it is not a pyrophoric solid and does not emit flammable gas on contact with water.

In a standard study (EEC Method A14), pyroxsulam did not exhibit any explosive properties. As such, it does not meet the criteria for classification as an explosive substance.

Finally, in a standard study (EEC Method A17), pyroxsulam ignited and charred, but did not burn to completion. As such, it is not classified as an oxidising solid.

3.1.3 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

In standard studies pyroxsulam failed to sustain combustion, did not exhibit any explosive properties and did not burn to completion once ignited. Experience in handling and use indicated that it is not a pyrophoric solid and does not release flammable gas on contact with water.

Comments received during public consultation

One Member State Competent Authority (MSCA) noted that information was available in the DAR on the solubility of pyroxsulam in organic solvents.

Additional key elements

Solubility in organic solvents (technical active substance):

Moderately soluble in n-heptane, n-octanol and xylene. Readily soluble in all other organic solvents tested. At 20°C the solubility (g/l) is as follows; n-heptane, <1; n-octanol 0.073; xylene, 0.0352; 1,2-dichloroethane, 3.94; methanol, 1.01; acetone, 2.79; ethyl acetate, 2.17.

Assessment and comparison with the classification criteria

There is no data to indicate that classification is warranted.

4 HUMAN HEALTH HAZARD ASSESSMENT

References are taken from the Draft Assessment Report (DAR) – Pyroxsulam - Volume 3, Annex B6: Toxicology and Metabolism – January 2012

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The following summary is derived from the Pesticide Assessment Report made for the review under Directive 91/414/EEC.

The toxicokinetics of pyroxsulam have been investigated in two species: rat and mouse.

In the rat study, pyroxsulam was shown to be rapidly absorbed with around 74-78 % of the dose being absorbed after administration with 10 mg/kg bw/day. The 1000 mg/kg bw/day was absorbed to a lesser extent. Of the tissues investigated, highest systemic levels occurred in the plasma, liver and kidney. Pyroxsulam was rapidly excreted in the urine and faeces (nearly 100 % of the absorbed dose within 48 h). Pyroxsulam was mostly excreted unchanged (85-90% of administered dose). The only identified metabolite was 2-desmethyl-XDE-742, which was present in both urine and faeces (at least 5 % of low dose administered). There were no differences in findings between the two labelling positions (labelled in the triazole or pyridine rings), nor between single or repeat dosing. Notably, there was no evidence for metabolic induction (no alteration in metabolism of pyroxsulam) as a result of repeat dosing with unlabelled pyroxsulam. The rapid and extensive excretion with very low levels in carcass at 38 h post dose (< 1% of administered dose) suggests there is little potential for accumulation.

In the mouse study, pyroxsulam was rapidly absorbed. After oral dosing with 10 mg/kg bw/day, about 60 % was absorbed; a lesser percentage was absorbed at 1000 mg/kg bw/day. Limited data indicate that liver concentrations of radiolabel rose to significantly higher levels in males than females. Radiolabel was cleared quickly from plasma, RBC and liver during the initial elimination phase ($t_{1/2}$ of 2-3h) and subsequently more slowly (especially from the liver) at the high dose. The slow elimination from the liver, will favour accumulation of pyroxsulam/metabolites in the liver on repeated dietary exposure. Of the limited tissues investigated at 72 h, highest systemic levels were in the liver.

Compared to male rats, at the plasma Cmax following an oral dose of 10 mg/kg bw, the concentration of radiolabel in the liver was slightly higher for male mice, but decreased to similar levels in both species at 2h and 48h. It is also notable that at 48h (only timepoint with data available) after dosing with 1000 mg/kg bw, the concentration in the liver of male rats (16.7 ug-eq./g) was slightly higher than in the male mice (8.2 ug-eq./g).

Pyroxsulam-derived radioactivity was rapidly and extensively excreted in urine and faeces (93-100 % within 24 h). Excretion was mainly in urine at 10 and 100 mg/kg bw, but mainly in faeces at 1000 mg/kg bw.

4.1.2 Human information

No information available

4.1.3 Summary and discussion on toxicokinetics

The toxicokinetics of pyroxsulam was investigated orally in rats (single and repeated administration) and mice (single dose only). Following single (rats and mice) and repeat administration (rat only), pyroxsulam was well absorbed. In rats, distribution was highest in the plasma, liver and kidney. Only a small proportion of pyroxsulam was metabolised. Excretion was via both the urine and faeces in rats and mainly via the urine in mice at the low dose and via the faeces at the high dose. There was no evidence of bioaccumulation.

4.2 Acute toxicity

Table 10: Summary table of relevant acute toxicity studies

Acute Oral		
Method	**LD_{50}**	**Observations and remarks**
OECD 423 (2001) GLP 6 female Wistar rats Single dose of 2000 mg/kg bw *Gamer and Leibold (2003a)*	> 2000 mg/kg bw	An initial dose (2000 mg/kg bw/day) was given to 3 fasted rats. As none died a further 3 rats were dosed in the same manner. No mortality or effects observed
Acute Inhalation		
Method	**LC_{50}**	**Observations and remarks**
OECD 403 (1981) GLP 5 F344 rats/sex Exposed nose only for 4 h to 5.12 mg/L (dust/aerosol) MMAD – 3.6 microns *Lowe (2007a)*	> 5.12 mg/L	There were no deaths or clinical signs of toxicity. Although most rats had lost weight by day 1 or 3 (post-exposure), all animals surpassed their pre-exposure weight by day 7 and continued to gain body weight through day 14. There were no treatment-related lesions at necropsy.
Acute Dermal		
Method	**LD_{50}**	**Observations and remarks**
OECD 402 (1987) GLP 5 Wistar rats/sex Single dose of 2000 mg/kg bw Vehicle: doubly distilled water Semi occlusive *Gamer and Leibold (2003b)*	> 2000 mg/kg bw	No mortality or adverse effects observed

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

An oral LD_{50} of > 2000 mg/kg bw/day was derived from a study conducted in rats.

4.2.1.2 Acute toxicity: inhalation

An inhalation 4 hr LC_{50} of > 5.12 mg/L was derived from a study conducted in rats.

4.2.1.3 Acute toxicity: dermal

A dermal LD_{50} of > 2000 mg/kg bw/day was derived from a study conducted in rats.

4.2.1.4 Acute toxicity: other routes

No information available

4.2.2 Human information

No information available

4.2.3 Summary and discussion of acute toxicity

See section 4.2.1

4.2.4 Comparison with criteria

Via the oral, inhalation and dermal routes, the LD_{50} values were higher than the respective guidance values (2000 mg/kg, 5 mg/l and 2000 mg/kg respectively); no classification is required.

4.2.5 Conclusions on classification and labelling

Not Classified: conclusive but not sufficient for classification

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity of Pyroxsulam in rats

The results of one guideline (OECD 423, 2001) and GLP compliant "acute toxic class" (limit dose) study (*Gamer and Leibold, 2003a*) was presented by the DS. This was conducted with six female Wistar/HanBrl:WIST(SPF) rats. Acute oral LD_{50} values for pyroxsulam (purity 98%) were greater than the limit dose of 2000 mg/kg bw. No mortality occurred. No clinical signs of toxicity were observed. The mean body weights in the treated groups increased throughout the study period. No macroscopic pathologic abnormalities were noted in the animals examined at the end of the observation period.

The DS did not propose classification for acute oral toxicity on the basis that no effects were seen in female Wistar rats in the study by Gamer and Leibold (2003a).

Acute inhalation toxicity of Pyroxulam in rats

The results of a single GLP and guideline (OECD 403, 1981) compliant , acute inhalation toxicity study was presented by the DS. All exposures were for 4 hours using five F344 rats/sex. The *Lowe (2007a)* study used a nose-only dynamic inhalation exposure system, to a time-weighted average chamber concentration of 5.12 mg pyroxsulam dust per liter of air. There were no deaths or clinical signs of toxicity at the highest attainable concentration of 5.12 mg/L /4h. The LC_{50} was > 5.12 mg/L /4h. There were no visible treatment-related lesions at necropsy. In addition there were no signs of respiratory irritation.

The DS did not propose classification for acute inhalation toxicity on the basis that no effects were seen in male and female F344 rats in the study by Lowe (2007a).

Acute dermal toxicity of Pyroxsulam

The results of one GLP and guideline (OECD 402, 1987) compliant , acceptable acute dermal toxicity study using 5 Wistar rats/sex was presented by the DS. The study by *Gamer & Leibold (2003b)* did not show mortality at 2000 mg/kg bw. No systemic clinical observations or skin effects were noted in the animals. No macroscopic pathologic abnormalities were noted in the animals examined at the end of the study. The LD_{50} was judged to be > 2000 mg/kg bw.

The DS did not propose classification for acute dermal toxicity on the basis that no effects were seen in male and female Wistar rats in the study by Gamer & Leibold (2003b).

Comments received during public consultation

One MSCA supported no classification for acute toxicity as proposed by the DS.

Assessment and comparison with the classification criteria

An oral LD_{50} of > 2000 mg/kg bw/day was derived from a study conducted in rats. According to CLP, LD_{50} values for acute oral toxicity > 2000 mg/kg bw do not warrant classification. RAC is in agreement with the DS that **no classification for acute oral toxicity is warranted for pyroxsulam.**

An inhalation 4 hr LC_{50} of > 5.12 mg/L was derived from a study conducted in rats. According to CLP, LC_{50} values for acute inhalation > 5 mg/L for dust/mist do not warrant classification. The RAC is in agreement with the DS that **no classification for acute inhalation is warranted for pyroxsulam.**

A dermal LD_{50} of > 2000 mg/kg bw/day was derived from a study conducted in rats. According to CLP, LD_{50} values for acute dermal toxicity > 2000 mg/kg bw do not warrant classification. RAC is in agreement with the DS that **no classification for acute dermal toxicity is warranted for pyroxsulam.**

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

No clinical signs or changes in organs were observed in any of the acute studies (table 10).

4.3.2 Comparison with criteria

Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure are classified in STOT-SE 1 or 2. Classification is supported by evidence associating single exposure to the substance with a consistent and identifiable toxic effect.

Classification in STOT-SE 3 is reserved for transient target organ effects and is limited to substances that have narcotic effects or cause respiratory tract irritation.

Since no clinical signs or changes in organs were observed (table 10), the criteria for STOT SE are not met

4.3.3 Conclusions on classification and labelling

Not Classified: conclusive but not sufficient for classification

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

Summary of the Dossier Submitter's proposal

There were no clinical signs or changes in organs observed in any of the acute studies described by the DS in the CLH report. The DS did not propose classification.

Comments received during public consultation

One MSCA supported no classification for STOT SE as proposed by the DS.

Assessment and comparison with the classification criteria

No comparison with the criteria is necessary. There were no clinical signs or changes in organs observed in any of the acute toxicity studies and the criteria for STOT SE are not met. Accordingly RAC agrees with the DS that **no classification for STOT SE** is warranted.

4.4 Irritation

4.4.1 Skin irritation

Table 11: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
OECD 404 (2002) GLP 0.5g of test substance moistened with distilled water and applied for 4 hours. 3 New Zealand rabbits Semi-Occlusive	Slight erythema (grade 1) was observed in all animals immediately and up to 1 hour after removal of the patch. No other cutaneous reactions were observed during the study. Mean scores over 24, 48 and 72 hours were 0 for erythema and oedema	Pyroxsulam showed slight transient irritation at the 1 hour time point only	Kaufmann and Leibold (2003a) and Kaufmann (2006a)

4.4.1.1 Non-human information

The skin irritation potential of pyroxsulam has been investigated in one standard guideline study in rabbits. Only slight transient irritation was observed.

4.4.1.2 Human information

No information available

4.4.1.3 Summary and discussion of skin irritation

The skin irritation potential of pyroxsulam has been investigated in one standard guideline study in rabbits. Slight transient irritation was observed at the 1 hr observation only.

4.4.1.4 Comparison with criteria

No oedema or erythema was observed over the time points relevant for classification (24, 48 and 72 hours); therefore, no classification for skin irritation is required.

4.4.1.5 Conclusions on classification and labelling

Not Classified: conclusive but not sufficient for classification

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The skin irritation potential of pyroxsulam was investigated in one standard guideline (OECD 404, 2002) and GLP compliant study in rabbits (*Kaufmann & Leibold, 2003a* and

original report amendment by *Kaufmann, 2006a*). 0.5 g of the test substance moistened with distilled water was applied for 4 hours to the intact skin of three New Zealand White rabbits, using a patch of 2.5 x 2.5 cm, which was covered with semi occlusive dressing. Only slight transient irritation (grade 1) was observed at the 1 hr observation time point. No other cutaneous reactions were observed during the study. Mean scores over 24, 48 and 72 hours for each animal were 0.0 for erythema and oedema. Reference was also made in the DAR to an initial test with the *in vitro* EpiDerm™ human skin model which showed non corrosivity for pyroxsulam. A summary page of the test results was included in the annex of the original study report by *Kaufmann & Leibold, (2003a)* and the results are detailed below under the key elements section. The DS did not propose classification.

Comments received during public consultation

One MSCA supported no classification for skin hazards as proposed by the DS.

Additional key elements

EpiDerm Corrosivity Test Results: in a non GLP test (table 1), pyroxsulam tested negative for its ability to directly reduce MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]). The results showed little effect on the viability of test tissues relative to the negative control. Pyroxsulam is non corrosive because it did not meet the criteria for corrosivity (i.e. viability at 3min: < 50% and / or 1 hour: < 15%) under the tested conditions.

MTT test results (indicator of tissue viability)

Test Article	OD_{570} tissue 1	OD_{570} tissue 2	OD_{570} mean	Viability (% of NC*)
Exposure: 3min				
negative control	2.108	2.057	2.083	100
pyroxsulam	1.998	1.963	1.980	**95**
positive control	0.351	0.320	0.335	16
Exposure: 1 hour				
negative control	1.925	2.130	2.028	100
pyroxsulam	1.857	2.146	2.002	**99**
positive control	0.196	0.199	0.198	10

*NC: negative control.

Assessment and comparison with the classification criteria

No oedema or erythema was observed over the time points relevant for classification (24, 48 and 72 hours); therefore, no classification for skin irritation is required. Accordingly RAC agrees with the DS that **no classification for skin corrosion/irritation** is warranted.

4.4.2 Eye irritation

Table 12: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
OECD 405 (2002) GLP 0.1 ml of test substance was applied for 1 hour. 3 New Zealand rabbits (application was a stepwise procedure starting with one animal and then two additional animals)	Slight conjunctival redness (grade 1) was observed in all animals 1 hour after application. This persisted in two animals up to 24 hours and in one animal up to 48 hours Grade 1 chemosis was observed in one animal between 1-24 hour Mean scores for each animal calculated over 24, 48 and 72 hours 0, 0 and 0 for corneal opacity and iris lesions 0.7, 0.3, 0.3 for redness of the conjunctiva 0, 0 and 0.3 for chemosis	Injected scleral vessels in a circumscribed area were noted in one animal after 24 hours	Kaufmann and Leibold (2003b) and Kaufmann (2006b)

4.4.2.1 Non-human information

The eye irritation potential of pyroxsulam has been investigated in a standard guideline study. No effects on the cornea or iris were noted. Effects on the conjunctivae were limited to mild erythema and oedema.

4.4.2.2 Human information

No information available

4.4.2.3 Summary and discussion of eye irritation

The eye irritation potential of pyroxsulam has been investigated in a standard guideline study. No effects on the cornea or iris were noted. Effects on the conjunctivae were limited to erythema and mild oedema.

4.4.2.4 Comparison with criteria

No effects in the iris or cornea were noted. The mean scores for each animal calculated over 24, 48 and 72 hours for erythema and oedema of the conjunctivae were less than the guidance value of 2. No classification is required.

4.4.2.5 Conclusions on classification and labelling

Not Classified: conclusive but not sufficient for classification

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The eye irritation potential of pyroxsulam was investigated in a standard guideline (OECD 405, 2002) and GLP compliant study (*Kaufmann & Leibold, 2003b*) using three New Zealand White rabbits (stepwise procedure starting with one animal and supplementing two additional animals). Approximately 1 hour after application, the treated eyes were rinsed with tap water. No effects on the cornea or iris were noted. Effects on the conjunctivae were limited to mild erythema and oedema and no single animal scored in excess of the CLP guidance trigger values (table below).

Mean values for ocular lesions 24, 48 and 72 hours after instillation

Animals	Corneal opacity	Iridial lesions	Conjunctival	
			Redness	Chemosis
1	0	0	0.7	0
2	0	0	0.3	0
3	0	0	0.3	0.3
CLP Criteria: Eye Irrit 2	≥ 1	≥ 1	≥ 2	≥ 2
CLP Criteria: Eye Irrit 1	≥ 3	> 1.5	na	na

Reference was also made in the DAR to an *in vitro* study using the chorio-allantoic membrane in incubated hen eggs (HET CAM test) where pyroxsulam did not produce changes indicative of severe eye irritation. A summary page was included in the annex of the original study report by Kaufmann & Leibold, (2003b) and the results are detailed below under the key elements section. In conclusion, the DS does not support classification.

Comments received during public consultation

One MSCA supported no classification for eye hazards as proposed by the DS.

Additional key elements

HET CAM Test Results

In a non GLP *in vitro* corrosion test (table below), using the chorio-allantoic membrane (CAM) in incubated hen eggs (HET CAM test), pyroxsulam did not show any effects on the CAM such as bleeding or protein denaturation. Pyroxsulam is non corrosive or irritating in this preliminary test.

HET CAM test results

Concentration	**Egg no. (exposure time)**	**Grading of final endpoints**	
		Haemorrhagia	***Coagulation***
Undiluted test substance	1 (5 min)	No effect	No effect
	2 (5 min)	No effect	No effect
	3 (5 min)	No effect	No effect

Assessment and comparison with the classification criteria

Criteria for Eye Irrit. 2: corneal opacity or iritis score ≥ 1 or conjunctival redness or edema score ≥ 2, in two out of three animals which fully reverse within the observation period of 21 days.

No effects in the iris or cornea were noted. The mean scores for each animal calculated over 24, 48 and 72 hours for erythema and oedema of the conjunctivae were less than the CLP guidance value of 2. RAC supports the DS conclusion that **no classification is warranted for eye corrosion / irritation**.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

This endpoint was not investigated directly; however, no signs of respiratory irritation were observed in the acute inhalation study (see section 4.2).

4.4.3.2 Human information

No information available

4.4.3.3 Summary and discussion of respiratory tract irritation

This endpoint was not investigated directly; however, no signs of respiratory irritation were observed in the acute inhalation study (see section 4.2).

4.4.3.4 Comparison with criteria

No signs of respiratory tract irritation were observed.

4.4.3.5 Conclusions on classification and labelling

Not Classified: Data lacking

4.5 Corrosivity

Table 13: Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference
Refer to table 11			

4.5.1 Non-human information

Pyroxsulam is not irritating to skin (see section 4.4)

4.5.2 Human information

No information available

4.5.3 Summary and discussion of corrosivity

Pyroxsulam is not irritating to skin (see section 4.4)

4.5.4 Comparison with criteria

No signs of corrosivity were observed in an *in vivo* skin irritation study.

4.5.5 Conclusions on classification and labelling

Not Classified: conclusive but not sufficient for classification

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 14: Summary table of relevant skin sensitisation studies

Species/Method	Doses	No. sensitised/total no.	Result	Reference
OECD 406 (1992) GLP Guinea-pig/Dunkin-Hartley 10 control animals 20 test animals Intradermal induction performed on day 0 and epicutaneous	Induction: Intradermal: 5 % pyroxsulam in 1 % CMC (carboxymethylcellulose) sodium solution in water Topical: 50 % pyroxsulam in 1 % CMC-solution in water Challenge: 25 % pyroxsulam in 1 % CMC-solution water Erythema and/or swelling observed following intradermal and topical induction.	Test: 24 h: 16/20 48 h: 15/20 Negative Control: 0/10 at 24 and 48 h Positive control: alpha-hexylcinnamaldehyde, techn. 85% showed test system was able to detect sensitizing compounds	Positive	Gamer and Leibold (2004)

induction on day 7. Challenge was 14 days after the epicutaneous induction.				

4.6.1.1 Non-human information

The skin sensitisation potential has been investigated in a standard maximisation study. Positive responses were observed in 16/20 animals at 24 hours and 15/20 animals at 48 hours compared to 0/10 in the control.

4.6.1.2 Human information

No information is available.

4.6.1.3 Summary and discussion of skin sensitisation

The skin sensitisation potential has been investigated in a standard maximisation study. Positive responses were observed in 16/20 animals (80%) at 24 hours and 15/20 (75%) animals at 48 hours compared to 0/10 in the control.

4.6.1.4 Comparison with criteria

A substance is classified in Category 1A where

a) there is a ≥ 30% response in animals receiving an intradermal induction dose of ≤ 0.1% or

b) there is ≥ 60% response in animals receiving an intradermal induction dose of > 0.1% and ≤ 1% in a GPMT.

A substance is classified in Category 1B where

a) there is a ≥ 30% to <60% response in animals receiving an intradermal induction dose of > 0.1% and ≤ 1% or

b) there is a ≥ 30% response in animals receiving an intradermal induction dose of > 1% in a GPMT;

where there is no information to suggest that classification in Category 1A should be considered,

The sensitisation response in the available Guinea-Pig maximisation study with pyroxsulam was 80%, with an intradermal induction of 5%. Whilst this meets the criteria for classification in Category 1B, it should be noted that a relatively high response (80%) was observed with an induction dose of 5% and no data are available from standard studies at lower induction concentrations. As such it could be that classification in Category 1A can not be excluded and a simple argument for classification in Category 1 can be made.

4.6.1.5 Conclusions on classification and labelling

Skin Sens 1; H317 May cause an allergic skin reaction

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The skin sensitisation potential of pyroxsulam was investigated in a standard GLP and guideline compliant (OECD 406, 1992) guinea pig Maximization Test based on the method of Magnusson and Kligman (*Gamer & Leibold, 2004*). After challenge, discrete or patchy to intense erythema were observed in most test group animals with swelling, scaling or severe scaling. Positive responses were observed in 16/20 animals (80%) at 24 hours and 15/20 animals (75%) at 48 hours compared to 0/10 in the control group following challenge with 25% pyroxsulam in 1% CMC solution in doubly distilled water. The intradermal induction concentration was 5%.

The DS proposes Skin Sens. 1 - H317 with no sub-categorisation on the basis of positive results from an M&K maximisation study with an intradermal induction of 5% pyroxsulam.

Comments received during public consultation

Two MSCA supported the classification proposal by the DS with no sub-categorisation.

Assessment and comparison with the classification criteria

According to Annex I, Section 3.4.2.2.1.1 of CLP, skin sensitisers shall be classified in Category 1 where data are not sufficient for sub-categorisation. However, according to Annex I, Section 3.4.2.2.1.2 of CLP, where data are sufficient, a refined evaluation on the basis of the occurrence or potency of the sensitising effect can allow the allocation of skin sensitisers into sub-category 1A (high frequency of occurrence, strong sensitisers), or sub-category 1B (a low to moderate frequency of occurrence, low to moderate potency).

The criteria for classification in category 1B for skin sensitisation on the basis of the M&K Guinea Pig Maximisation Test are as follows: If a test substance is present at > 1% for intradermal induction and the incidence of sensitisation is ≥ 30%.

In the *Gamer & Leibold, (2004)* study there is an 80% response rate with an intradermal induction of 5% pyroxsulam. The criteria seem to be satisfied for Skin Sens 1B – H317. However, it should be noted that there are no data available at lower induction concentrations. Thus, because of the relatively high response (80%) observed with an induction dose of 5%, classification in sub-category 1A cannot be excluded. Data are not available to conclude if the criteria for Skin Sens 1A are met (substance present at > 0.1% to ≤ 1% for intradermal induction with an incidence of sensitisation ≥ 60%). Therefore, a lower concentration of pyroxsulam in the GPMT may still have a high response rate in excess of the trigger value of 60% (and in doing so may satisfy the case for 1A), but this presumption has not been tested. In accordance with the Guidance on the Application of the CLP Criteria (Annex I: 3.4.2.2.1.1), classification as a Category 1

skin sensitiser with no sub-categorisation is considered appropriate in this case.

In conclusion, an evaluation of the sensitising response observed for pyroxsulam does not allow classification into sub-categories and accordingly RAC is in agreement with the DS and concludes that **classification as Skin Sens. 1 – H317 is warranted**.

4.6.2 Respiratory sensitisation

Table 15: Summary table of relevant respiratory sensitisation studies

Method	Results	Remarks	Reference
Not applicable			

4.6.2.1 Non-human information

No data are available.

4.6.2.2 Human information

No data are available.

4.6.2.3 Summary and discussion of respiratory sensitisation

No data are available.

4.6.2.4 Comparison with criteria

No data are available.

4.6.2.5 Conclusions on classification and labelling

Not classified: Data lacking.

4.7 Repeated dose toxicity

Information on repeated dose toxicity is available from short-term dietary studies in rats, mice and dogs. A short-term dermal study in rats is also available.

Table 16: Summary table of relevant repeated dose toxicity studies

Method	Dose Levels	Observations and Remarks	Reference
Rat 28-day study Dietary OECD 407 (1995) GLP 5 F344 **rats**/sex/dose	0, 10, 100, 500 or 1000 mg/kg bw/day Actual doses received were in excess: 0, 11.8, 120, 583 and 1165 mg/kg bw/day in males 0, 11.6, 112, 563 and 1140 mg/kg bw/day in females Dose level relevant for classification (determined from the guidance value for 90-day rat study) - 300 mg/kg bw/d	1000 mg/kg bw/day Perineal urine soling of 1 female, 4% ↓ bodyweight gain (both sexes), ↓ serum ALT in females (not statistically significantly) 500 mg/kg bw/day Perineal urine soiling of 2 females 100 and 10 mg/kg bw/day No treatment related effects observed NOAEL of 1000 mg/kg bw/day in both sexes	Stebbins and Day (2001)

Method	Dose Levels	Observations and Remarks	Reference
Rat 90-day study Dietary OECD 408 (1998) Main treatment group: 10 F344 rats/sex/dose 28-day recovery group: 10 F344 rats/sex/dose	0, 10, 100 or 1000 mg/kg bw/day Dose level relevant for classification (guidance value for 90-day rat study) - 100 mg/kg bw/d	1000 mg/kg bw/day 3 males and 15 females showed perineal urine soiling 6/15% ↓ bodyweight gain in males/females 9% ↑ relative liver weight in males 4% ↓ serum ALT 37% ↑ serum cholesterol in males 20% ↑ in urine volume (ml) and ↓ protein (mg/dL) 100 mg/kg bw/day 4% ↓ bodyweight gain in females 10 mg/kg bw/day No treatment related effects observed Recovery group: effects had completely recovered or showed signs of recovery during the 28-day recovery period NOAEL of 100 mg/kg bw/day based on reduced bodyweight at 1000 mg/kg bw/day	Stebbins, Dryzga, Brooks, Thomas (2003)
Mouse 90-day study Dietary OECD 407 (1998) GLP 10 CD-1 mice/sex/dose	0, 10, 100, 1000 mg/kg bw/day Dose level relevant for classification (determined from the guidance value for 90-day rat study) - 100 mg/kg bw/d	1000 mg/kg bw/day 25% ↑ in male and female bodyweight ↑ food consumption in males 22/ 30% ↑ serum cholesterol in males/females (but within the historical control range) 18.3/ 8 % ↑ absolute liver weight in males/females, 12.3/5% ↑ relative liver weight in males/females 100 and 10 mg/kg bw/day No treatment related effects observed The NOAEL for males is 100 mg/kg bw/day based on increased liver weight at 1000 mg/kg bw/day The NOAEL for females is 1000 mg/kg bw/day	Johnson, Brooks, Drygza (2003)

Method	Dose Levels	Observations and Remarks	Reference
Dog 28-day study Dietary US EPA QPPTS 870.3700 GLP (except for histological processing and examination) 2 Beagle dogs/sex/dose Minimal quantitative detail is included due to low animal number	0, 0.3, 1 and 3% in the diet Equivalent to 0, 85, 421, 868 mg/kg bw/day in males and 0, 169, 333 and 1004 mg/kg bw/day in females Dose level relevant for classification (determined from the guidance value for 90-day rat study) - 300 mg/kg bw/d	3% dose level Slight ↓ bodyweight in males and females 79% and 37% ↑ serum cholesterol in each female dog ↑ absolute and relative liver weight in males 1% dose level ↑ absolute and relative liver weight in males 0.3% dose level No treatment related effects observed No NOAEL derived due to small group sizes	Merriman (2002)
Dog 90-day study Dietary OECD 409 (1998) GLP 4 Beagle dog/sex/dose	0, 0.03, 0.3 and 3% Equivalent to 0, 11, 91 and 884 mg/kg bw/day in males and 0, 10, 99 and 1142 mg/kg bw/day in females Dose level relevant for classification (determined from the guidance value for 90-day rat study) - 100 mg/kg bw/d	3% dose level ↓bodyweight in both sexes, 34/31% ↓ bodyweight gain in males/females (female reduction mainly due to one female who didn't gain any weight over period), slightly ↓ food consumption in males from week 3 27% ↑serum cholesterol in females (within historical control range), 23% ↑alkaline phosphatase levels in females (mainly due to an increase in one female) 11% ↑ in relative liver weight in males, 28% ↑ in absolute liver weight in females and 32% ↑ in relative liver weight in females. Slight panlobular hepatocellular hypertrophy in female livers 0.3 and 0.03% doses levels No treatment related effects noted A NOAEL of 0.3% (99 and 99 mg/kg bw/day in males and females, respectively) based on reduced bodyweight gain in males and effects in the liver in females	Stebbins and Baker (2003)
Dog 1 year study Beagle dogs OECD 452 (1981) GLP Four/sex/dose	0, 0.05, 0.3 and 2% 0, 13, 93 and 630 mg/kg bw/day in males and 0, 17, 89 and 589 mg/kg bw/day	2% dose level ↓ 9-11% decrease in red blood cell parameters in females. NB, the RBC parameters in the high dose group were slightly lower than controls at the start of the study 42/100% ↑ serum cholesterol in males/females. For males, the 12-month high dose value (reported here) exceeded the historical control, whereas none of the	Stebbins and Dryzga (2004)

Method	Dose Levels	Observations and Remarks	Reference
	in females Dose level relevant for classification (determined from the guidance value for 90-day rat study) – c.a. 25 mg/kg bw/d	individual values exceeded the range of values seen in the concurrent controls 145/38% ↑ alkaline phosphatase in males and females 23/ 20% ↑ absolute liver weight in males/females, 20/ 23% ↑ relative liver weight in males/females 0.3 and 0.05% dose levels No treatment related effects A NOAEL of 0.3% (93 mg/kg bw/day in males and 89 mg/kg bw/day in females) was derived based on increased liver weight	
14-day dermal study No guideline – range finding study GLP (but no QA)	0, 1000 mg/kg bw/day	1000 mg/kg bw/day No treatment related findings observed A NOAEL of 1000 mg/kg bw/day	Kaspers (2004)

NB: The values for NOAEL and LOAEL are provided for information only: they have already been agreed at a PRAPER expert meeting. ↓= decrease compared to control. ↑/↓ = increased/decreased compared to control.

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Sub-acute toxicity

Information on sub-acute toxicity is available from a 28-day study in rats and a 28-day and a 90-day study in dogs.

There were no adverse effects observed below the relevant guidance values for classification (300 mg/kg bw/day) in any study. At dose levels above the cut-off (~1000 mg/kg bw/day) effects included reduction in bodyweight and slight changes in clinical chemistry parameters (ALT and ↑ serum cholesterol)[1]. In both dog studies, liver weights were also increased, and were accompanied by associated histopathological changes in the 90-day study.

Sub-chronic toxicity

Information on sub-chronic toxicity comes from a 90-day study in rats, a 90-day study in mice and a one-year study in dogs.

There were no adverse effects observed below the relevant guidance values for classification (100 mg/kg bw/day). At dose levels above the guidance values (> 589 mg/kg bw/day) effects including

[1] In a number of rat studies, perineal urine staining was observed. This effect was not considered adverse and is not discussed further.

reductions in bodyweight (rats and dogs only), slight, non-adverse, changes in clinical chemistry (↑ alkaline phosphatase and ↑ serum cholesterol) and increased liver weights were observed in all species unless specified.

Chronic toxicity

Information on chronic toxicity comes from a study in rats and a study in mice (see section 4.10).

In both studies, effects were only noted at the limit dose (1000 mg/kg bw/day) and were comparable to those observed in the other studies (bodyweight, liver effects, and clinical chemistry changes). The only exception to this was an increase in kidney weight (relative and absolute) observed in mice.

4.7.1.2 Repeated dose toxicity: inhalation

No information available.

4.7.1.3 Repeated dose toxicity: dermal

Limited information on sub-acute toxicity is available from a 14-day study in rats. In this study no effects were observed at the limit dose. No classification is warranted.

4.7.1.4 Repeated dose toxicity: other routes

No information available.

4.7.1.5 Human information

No information available.

4.7.1.6 Other relevant information

Not applicable

4.7.1.7 Summary and discussion of repeated dose toxicity

See sections 4.7.1.1 and 4.7.1.3

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

Information on pyroxsulam is available from oral studies in rats, mice and dogs. There is also information available from a dermal study in rats.

The rat data show that there are no serious adverse effects of pyroxsulam below the guidance values (300 mg/kg bw/day in a 90-day study in rats) for classification, with effects occurring only at higher dose levels (reduced bodyweight and liver effects). The mouse and dog data confirm pyroxsulam is of low toxicity. The main adverse effects were reduced bodyweight (dog) and effects on the liver (both dogs and mice).

The results of a 14-day dermal range-finding study showed no effects up to the limit dose.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

The available information indicates that classification for repeated dose toxicity is not warranted as no significant adverse effects were observed below the guidance values for classification.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Not Classified: conclusive but not sufficient for classification

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

Summary of the Dossier Submitter's proposal

The DS evaluated a variety of sub-chronic and chronic studies from rats, dogs and mice, including one short term (14 day) repeated dose dermal toxicity study in rats and presented a detailed summary of the effects in table 16 of the CLH report. A summary of the NOAELs and LOAELs from these studies is presented in the Table below. In addition, details are also provided from the chronic, oncogenicity studies in rats and mice and the reproductive toxicity studies in rats and rabbits.

In the dietary studies, the main target organ was the liver (increased liver weight and increased serum cholesterol were observed in all species tested). None of the studies support classification for STOT RE:

1. the effects are not sufficiently severe,
2. the effects are shown to be reversible, and
3. there were no adverse effects observed in any study below the highest relevant guidance values for classification (Cat 2: 300 mg/kg bw/day in the case of a 28-day study) in the CLP guidance.

Summary of repeat dose toxicity studies with pyroxsulam.

Study	NOAEL	LOAEL	[1]Effects at LOAEL	Reference
Oral studies:				
[2]**14-day dermal rat**	Males / Females: 1000 mg/kg bw/d	>1000 mg/kg bw/d	No adverse effects.	Kaspers (2004)
28-day dietary Rat OECD 407, GLP Strain: F344	Males: 1165 mg/kg bw/d	>1165 mg/kg bw/d	No adverse effects at highest dose tested.	Stebbins and Day (2001)
	Females: 1140 mg/kg bw/d	>1140 mg/kg bw/d		
90-day dietary Rat	Males: 1000 mg/kg bw/d	>1000 mg/kg bw/d	No adverse effects at highest dose tested.	Stebbins *et al* (2003)

OECD 408, GLP Strain: F344	Females: 100 mg/kg bw/d	1000 mg/kg bw/d	BW gain reduced 15% at top dose. Effects reversed during 28-day recovery period.	
1-Year dietary Rat (chronic neurotoxicity) OECD 424, GLP Strain: F344	Males / Females: 1000 mg/kg bw/d	>1000 mg/kg bw/d	No adverse effects at highest dose tested.	Maurissen *et al*., (2005)
2-Year chronic dietary Rat OECD 453, GLP Strain: F344	Males: 1000 mg/kg bw/d	>1000 mg/kg bw/d	No adverse effects	Stebbins & Brooks (2005, 2008 revision)
	Females: 1000 mg/kg bw/d	>1000 mg/kg bw/d	No adverse effects	
Multigeneration Rat (reprotoxicity) OECD 416, GLP Strain: CD	Males / Females: 1000 mg/kg bw/d	>1000 mg/kg bw/d	No adverse effects at highest dose tested.	Carney *et al* (2005)
	Developmental : 1000 mg/kg bw/d	>1000 mg/kg bw/d		
Developmental Rat (reprotoxicity) OECD 414, GLP Strain: CD	Maternal: 1000 mg/kg bw/d	>1000 mg/kg bw/d	No adverse effects at highest dose tested.	Carney & Tornesi (2005)
	Developmental : 1000 mg/kg bw/d	>1000 mg/kg bw/d		
Developmental Rabbit (reprotoxicity) **OECD 414, GLP** **Strain: NZW**	Maternal: 300 mg/kg bw/d	>300 mg/kg bw/d	No adverse effects at highest dose tested.	Sloter (2005b)
	Developmental : 300 mg/kg bw/d	>300 mg/kg bw/d		
90-day dietary Mouse OECD 408, GLP Strain: CD1	Males: 100 mg/kg bw/d	1000 mg/kg bw/d	Increased liver weight (12-18% relative - absolute)	Johnson, Books and Dryzga (2003)
	Females: 1000 mg/kg bw/d	>1000 mg/kg bw/d	No adverse effects	
18-Month chronic dietary Mouse OECD 451, GLP Strain: CD-1	Males: 100 mg/kg bw/d	1000 mg/kg bw/d	Rel. liver wt ↑ 32% Abs. liver wt ↑ 26% foci of altered hepatocytes ↑	Johnson *et al* (2005)
	Females: 1000 mg/kg bw/d	>1000 mg/kg bw/d	No adverse effects	
[3]**28-day dietary Dog** US EPA 870.3700, GLP	Males: 868 mg/kg bw/d	> 868 mg/kg bw/d	No adverse effects, few animals (2 animals per dose per sex).	Merriman (2002)
	Females: 1004 mg/kg bw/d	> 1004 mg/kg bw/d		

90-day dietary Dog OECD 409, GLP	Males: 91 mg/kg bw/d	884 mg/kg bw/d	BW gain ↓ 34%. Rel liver wt ↑ 13%	Stebbins and Baker (2003)
	Females: 99 mg/kg bw/d	1142 mg/kg bw/d	BW gain ↓ 31%. Rel liver wt ↑ 33%	
1-Year dietary Dog OECD 452, GLP	Males: 93 mg/kg bw/d	620 mg/kg bw/d	Rel. liver wt ↑ 22% Abs. liver wt ↑ 24%	Stebbins and Dryzga (2004)
	Females: 89 mg/kg bw/d	589 mg/kg bw/d	Rel. liver wt ↑ 23% Abs. liver wt ↑ 20%	

[1] primary effects observed at the LOAEL.

[2] supplementary study: there were no investigations of haematology, clinical chemistry, organ weights, gross pathology or histopathology.

[3] supplementary study only, effects (loss of body weight, increased liver weight, increased serum cholesterol) at high dose (868 – 1004 mg/kg bw/day) consistent with subsequent 90-day and 1-year studies with larger numbers of dogs.

The rat data showed that there were no serious adverse effects of pyroxsulam below the guidance values (300 mg/kg bw/day in a 90-day study in rats) for classification, with effects occurring only at higher dose levels, typically at the limit dose (reduced bodyweight and liver effects and perineal soiling, mainly in female rats - regarded as substance-related but not adverse). The mouse and dog data also confirmed that pyroxsulam is of low toxicity. The main adverse effects were reduced bodyweight (dog) and effects on the liver in all species tested (increased liver weight and increased serum cholesterol). There was no evidence of functional disturbances in any organ system or significant impacts on the health of the tested animals. The 90-day rat study by Stebbins *et al*. (2003) included a 28-day recovery group which showed near complete recovery of liver weight and cholesterol to pretreatment levels. The chronic rat and mouse studies also support the observed low toxicity of pyroxsulam and show perineal soiling in both sexes with no corresponding histopathological urinary tract effects and an absence of alterations in urinalysis parameters.

Comments received during public consultation

Only one comment from one Member State was received for this specific endpoint, supporting the no-classification proposal for STOT RE.

Assessment and comparison with the classification criteria

The oral guidance cut-off values for a classification for STOT RE in category 2 under CLP are: ≤ 300 mg/kg bw/day from subacute studies on rat (28 days), ≤ 100 mg/kg bw/day from subchronic studies on rat (90 days), ≤ 25 mg/kg bw/day from one year studies and ≤ 12.5 mg/kg bw/day from long term studies. If dermal studies are used then the cut-off values are 2-fold greater.

As described by the DS under section 4.7.1 of the CLH report, there were no adverse effects observed below the relevant guidance values for classification. RAC considers that **no classification for specific target organ toxicity after repeated exposure is warranted**.

4.9 Germ cell mutagenicity (Mutagenicity)

Table 17: Summary table of relevant in vitro and in vivo mutagenicity studies

In Vitro Data			
Method	**Organism/strain**	**Concentrations tested**	**Result**
Ames OECD 471 (1997) GLP Engelhardt and Leibold (2003)	*Salmonella strains* TA 1535, TA 100, TA 1537 and TA 98 *E.Coli* WP2 urvA	0-5000 µg/plate with and without S9 Vehicle dimethylformamide	Negative Precipitation was observed at 2500 µg/plate and above Depending on strain, a bacteriotoxic effect was observed between 750-5000 µg/plate Positive controls responded as expected
In vitro cytogenetic study OECD 473 (1997) GLP Schisler (2006)	Rat lymphocytes	0-200 µg/plate (top dose determined by solubility in the vehicle [DMSO], not cytotoxicity)	Negative No cytotoxicity or precipitation was observed No evidence of polyploidy Positive controls responded as expected
Mammalian cell gene mutation assay OECD 476 GLP Schisler and Grundy (2006)	Chinese hamster ovary (CHO) cells	0-200 µg/plate (top dose determined by solubility in the vehicle DMSO not cytotoxicity)	Negative No cytotoxicity or precipitation was observed Positive controls responded as expected
In vivo Data			
Method	**Organism/strain**	**Concentrations tested**	**Result**
Bone marrow micronucleus assay OECD 474 (1997) GLP Oral gavage (administered once on two consecutive days) Sacrificed 24 h after second dose Spencer and	CD-1 Mice 6 males/dose	0, 500, 1000 and 2000 mg/kg bw/day in 0.5% w/v methylcellulose	Negative No clinical signs or effects on bodyweight were observed No change in the % PCE values observed between treated and controls The positive control responded as expected

In Vitro Data			
Method	**Organism/strain**	**Concentrations tested**	**Result**
Grundy (2004)			
Unscheduled DNA Synthesis (UDS) Assay OECD 486 (1997) GLP Sacrificed 12-14h or 2-4 h after dosing Oral gavage Beevers (2006)	CD-1 mice 6 males/dose Hepatocytes from 3 animals/dose treated with [^{3}H] thymidine for 4 h	0, 1000 and 2000 mg/kg bw/day in 0.5% w/v methylcellulose	Negative No clinical signs of toxicity were observed The positive controls responded as expected

4.9.1 Non-human information

4.9.1.1 In vitro data

The genotoxicity of pyroxsulam has been investigated in an Ames test, an *in vitro* cytogenetics study and an *in vitro* mammalian cell gene mutation study. Positive controls were included in all assays and showed the expected responses. The result of all assays was negative.

4.9.1.2 In vivo data

The genotoxicity of pyroxsulam has been investigated *in vivo* in a mouse micronucleus study and an unscheduled DNA synthesis (UDS) study in mouse livers. The results of both studies were negative. No deaths or cytotoxicity was observed in either study. This is not considered to be a problem as toxicokinetic studies have shown pyroxsulam to be well distributed. In addition, with regards the UDS study, the liver has been identified as the target organ for this substance.

4.9.2 Human information

No information available

4.9.3 Other relevant information

Not applicable

4.9.4 Summary and discussion of mutagenicity

Data indicate pyroxsulam is not mutagenic *in vitro* or *in vivo*.

4.9.5 Comparison with criteria

Data indicate pyroxsulam is not mutagenic *in vitro* or *in vivo* and classification as a germ cell mutagen is not warranted.

4.9.6 Conclusions on classification and labelling

Not Classified: conclusive but not sufficient for classification

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Introduction

The DS reported that pyroxsulam has been tested in several *in vitro* and *in vivo* studies. The CLH report details each specific study in table 17. According to the 2012 pesticide DAR submitted to EFSA, pyroxsulam does not contain any structural alerts for potential DNA reactivity according to the model of Tennant and Ashby (1991).

Results* – In Vitro *Tests

The genotoxicity of pyroxsulam was investigated in an Ames test, an *in vitro* cytogenetics study and an *in vitro* mammalian cell gene mutation study. Positive controls were included in all assays and behaved as expected. The result of all assays was negative (Table below).

esults* – In Vivo *Tests

The genotoxicity of pyroxsulam was investigated *in vivo* in a mouse micronucleus study and an unscheduled DNA synthesis (UDS) study in mouse livers. The results of both studies were negative. No deaths or cytotoxicity was observed in either study (Table below). There was no evidence of toxicity to the bone marrow in the mouse micronucleus study. Exposure of the bone marrow to pyroxsulam was assumed based on ADME and toxicokinetics studies with radiolabelled pyroxsulam (which indicated that pyroxsulam was well absorbed orally with very limited subsequent metabolism).

Negative results were obtained in all studies with pyroxsulam. The two *in vivo* assays were performed with male CD-1 mice (the same sex and strain as in the mouse carcinogenicity study). There is no evidence of genotoxicity for pyroxsulam.

Summary of Genotoxicity tests with Pyroxsulam

Study	Result	Methods and acceptability	Reference
***In vitro* studies:**			
Bacterial mutagenicity	negative	GLP, OECD 471 (1997), acceptable *Salmonella* Strains: TA1535, 100, 1537, 98 Other: *E. coli* WP2 uvrA strain	Engelhardt & Leibold (2003)
Mammalian cell mutagenicity	negative	GLP, OECD 476 (1997), acceptable CHO/HPGRT	Schisler & Grundy (2006)
Clastogenicity	negative	GLP, OECD 473 (1997), acceptable Rat lymphocytes	Schisler (2006)
***In vivo* studies:**			
UDS	negative	GLP, OECD 486 (1997), acceptable Male mouse (CD-1) hepatocytes	Beevers (2006)
Micronucleus	negative	GLP, OECD 474 (1997), acceptable Male mouse (CD-1) bone marrow (short term)	Spencer & Grundy (2004)

Comments received during public consultation

One Member State commented supporting no classification for mutagenicity.

Assessment and comparison with the classification criteria

No human data are available for pyroxsulam, therefore a classification with Muta. 1A is not applicable. Pyroxsulam is negative in acceptable *in vitro* tests and *in vivo* somatic cell mutagenicity guideline tests in mammals. Data is not available for the induction of mutagenic effects in germ cells (a criterion for Category 1B). Overall, RAC agrees with the DS that the data **do not support classification for germ cell mutagenicity**.

4.10 Carcinogenicity

Table 18: Summary table of relevant carcinogenicity studies

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Two year chronic toxicity/carcinogenicity and chronic neurotoxicity study OECD 453 (1981) GLP Oral (dietary) 65 Fischer 344 rats/sex/dose 10 rats/sex/dose were necropsied at 1 year (chronic toxicity group). Of these, 5/rats/sex were shared with the group below In addition to the 5 rats above, a further 5 rats/sex/dose were necropsied at 1 year (chronic neurotoxicity group) 50 rats/sex/dose were fed diets up to two years and necropsied (oncogenicity group) Stebbins and Brooks (2005) and Stebbins and Brooks (2008) – revised report	0, 10, 100 and 1000 mg/kg bw/day	***Non-neoplastic effects*** *Mortality* No substance related effect on survival of female rats (2 year mortality was 20-24%). In males, mortality was slightly higher during the last 5 weeks of the study at 100 (44% at termination) and 1000 (52% at termination) mg/kg bw/day compared to the control (34%). *Clinical signs of toxicity* Incidence of perineal urine soiling was increased in both sexes of the 100 and 1000 mg/kg bw/day treatment groups. *Bodyweight* There were no effects observed in males. Female body weight gain of the 1000 mg/kg bw/day dose group was 8-10% ↓ over the two years. *Feed consumption* No effect in males Food consumption in females was statistically lower than controls between days 8 to 84. *Haematology* Minimally lower mean red blood cell counts in both sexes at 1000 mg/kg bw/day. Statistically significant at 6 months only and did not progress throughout the study. *Clinical Chemistry* Males at 1000 mg/kg bw/day had 36-38% ↓ ALT levels at 6 and 12 months and a 22-33% ↑ serum cholesterol at 3, 6 and 12 months *Urinalysis* 23/33% ↑ urine volume in males/females at 24 months in the 1000 mg/kg bw/day *Organ weights* 4.1/6.1% ↑ absolute liver weight in males/females and 8.8/10.9% ↑ relative liver weight in males/females of the 1000 mg/kg bw/day group *Gross pathology* No treatment related findings observed *Histopathology* ↓ incidence and/or severity of basophilic foci of altered hepatocytes in females given 1000 mg/kg bw/day (12 and 24 months) and in males given 1000 mg/kg bw/day (24 months) Slight ↑ erosion/ulceration of the glandular stomach and of diffuse hyperplasia of the non-glandular stomach in males of 1000 mg/kg bw/day (NB. Disparity between these findings and those of gross pathology where no increase in these

<table>
<tr><th>Method</th><th>Dose levels</th><th>Observations and remarks
(effects of major toxicological significance)</th></tr>
<tr><td></td><td></td><td>types of effect were observed).
<u>Neoplastic effects (key findings only)</u>
Leukemia, large granular lymphocyte (LGL), malignant, primary
<table>
<tr><td>Dose (mg/kg bw/day)</td><td>0</td><td>10</td><td>100</td><td>1000</td><td>Historical control</td></tr>
<tr><td>Male</td><td>20/50</td><td>21/50</td><td>28/50 (56 %)</td><td>29/50 (58 %)</td><td>Contemporaneous controls (2002-2005)
11/50, 18/50, 19/55, 17/50, 12/50
<u>(Older controls: 1992-1999</u>
9/50, 20/50)</td></tr>
<tr><td>female</td><td>12/50</td><td>6/50</td><td>8/50</td><td>11/50</td><td>Contemporaneous controls (2002-2005)
6/50, 9/50, 8/55, 12/50, 11/50
<u>(Older controls: 1992-1999</u>
14/50, 8/50, 14/50)</td></tr>
</table>
Other neoplastic findings have been summarized in the text below.
A NOAEL of 1000 mg/kg bw/day was derived for carcinogenicity. A NOAEL of 100 mg/kg bw/day was derived for non-neoplastic effects.</td></tr>
<tr><td>Eighteen month Dietary oncogenicity study
OECD 451 (1981)
GLP
Oral (dietary)
50 CD 1 mice/dose/sex
Johnson, Dryzga and Yano (2005)</td><td>0, 10, 100 or 1000 mg/kg bw/day</td><td><u>Non-neoplastic effects:</u>
Mortality
No substance related mortality. Mortality rates at the end of the study in control, 10, 100 and 1000 mg/kg bw/day were, respectively;
22, 20, 20 and 24% (males)
22, 28, 20 and 20% (females)
Clinical signs
There were no substance-related clinical signs
Bodyweight
There were treatment-related effects on bodyweight gain
Food consumption
Slight increases in food consumption were noted in males at 100 and 1000 mg/kg bw/day
Organ weights
25/32% ↑ absolute/relative liver weight of males at 1000 mg/kg bw/day</td></tr>
</table>

<table>
<tr><th>Method</th><th>Dose levels</th><th>Observations and remarks
(effects of major toxicological significance)</th></tr>
<tr><td></td><td></td><td>
10/12% ↓ absolute kidney weight and 6/10% ↓ relative kidney weights at 1000 mg/kg bw/day
Gross necropsy
The number of male mice with one or more liver nodules was slightly ↑ at 1000 mg/kg bw/day
Histopathology
↑ incidence of foci of altered hepatocytes observed in males at 1000 mg/kg bw/day (12 mice compared to 2 in the controls)

<u>Neoplastic effects:</u>
<table>
<tr><th>Dose</th><th>0</th><th>10</th><th>100</th><th>1000</th><th>Historical control</th></tr>
<tr><td colspan="6">Male mice</td></tr>
<tr><td>No of male mice with hepatocellular adenomas</td><td>5/50
(10%)</td><td>13/50
(26%)</td><td>9/50
(18%)</td><td>14/50
(28%)</td><td>a) 4-18%/ (2/50-9/50)
a2) 8-24% (4/50-12/50)
b) 1.4-20%</td></tr>
<tr><td>No of male mice with hepatocellular carcinomas</td><td>1/50
(2%)</td><td>0/50
(0%)</td><td>2/50
(4%)</td><td>4/50
(8%)</td><td>a) 0- 4% (0/50 – 2/50)
a2) 0-4% (0/50-2/50)
b) 1.6-15%</td></tr>
</table>

NOAEL could not be derived.
</td></tr>
</table>

a) Dow: studies necropsied Dec-2001- May 2001 (50 males/control), plus one study completed May 2007 and Notifier confirmed there were no multiple hepatocellular tumours in 50 male control mice).
a2) Dow: information from studies conducted between 2007 and 2012.
b) HCD in the Crl:CD1 mouse published by Charles River Labs (published paper M Gilkins and C. Clifford, March 2010): 13 studies initiated between 2002 – 2006. Includes studies of 78-104 weeks in duration (50-110 animals/study). An incidence of hepatocellular carcinoma of 6/60(10%) and 9/60 (15%) was observed in two 78 week studies. .

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Information is available from a carcinogenicity study in rats and mice.

Rats

In the rat study, neoplastic changes were observed in the haematopoietic/lymphoid system, liver, thyroid, and adrenals. These are discussed individually below:

Haematopoietic/lymphoid system

Males receiving 100 (28/50) or 1000 (29/50) mg/kg bw/day had slightly higher incidence of large granular lymphocyte (LGL Fischer rat) leukaemia[2] than in the controls (20/50). These increases were not statistically significant and there was no evidence for an early onset of LGL leukaemia in rats exposed to pyroxsulam (Stebbings and Brooks, 2008).

Historical control information

The incidence in the top two dose groups in males was outside the historical control range of dietary or oral gavage toxicity studies performed in this laboratory both contemporaneously (studies initiated: 2002-2005) and in the past (studies initiated: 1992-1995). However, the control incidence was also higher or equal to that of the historical controls too.

The incidence did fall within the NTP pre-1995 historical control range (32-74%), and was just outside the NTP post-1995 historical control range (30 to 54% based on 5 dietary studies). These historical control ranges suggest this type of tumour has a high spontaneous rate in Fischer rats. In addition, it is possible the diet may also influence tumour incidence with a higher top range observed with the pre-1995 diet (which was similar to the diet used in this study) than with the post-1995 diet (lower protein and higher fibre). However, on its own this assertion is not conclusive and since the incidence in the top two treatment groups was higher than both the concurrent controls and laboratory historical control data the tumours cannot be dismissed on this basis alone.

Dose response considerations

The incidence of LGL leukaemia in the 100 and 1000 mg/kg bw/day dose groups was similar. This was somewhat surprising given that the high dose group was ten times that of the mid dose group. Failure to see a dose-related increase in tumour incidence raises doubt that the tumours are treatment related.

Information from the repeated dose studies on the target organs

No substance-related increase in white blood cell count or substance-related changes in differential white blood count in male rats was observed. Nor was there any histological evidence that lymphoid tissues/organs were a target organ for pyroxsulam.

[2] Other names for this type of leukaemia include: mononuclear cell leukaemia; Fischer rat leukaemia and monocytic leukaemia.

Conclusion

Overall, an increase in the incidence of LGL leukaemia was observed at the top two doses in one sex (males). However, since, the control values were at the top of the historical control range; this is a relatively common tumour in Fischer rats; the increase was not statistically significant, the incidence was similar at both mid and high dose even though there was a 10-fold difference in dose level; and there was no evidence from repeated dose studies of effects in relevant organs (e.g. white blood cells, spleen liver, lungs, thymus, lymph glands), the increase in tumour incidence is not considered treatment related.

Additional tumour types in rat not summarised in the table:

Liver

A slight increase in the incidence of hepatocellular adenomas was observed in males treated with 1000 mg/kg bw/day (1/50, 3/50, 3/50 and 4/50 in controls to high dose). This increase was within the historical control ranges for dietary or oral gavage toxicity studies performed contemporaneously in this laboratory (Historical control range: 1-6 hepatocellular adenomas) and therefore was not considered treatment related. No increase was noted in females.

Thyroid

A slight increase in the incidence of parafollicular cell adenomas was observed in females treated with 1000 mg/kg bw/day (2/50, 2/10, 2/12 and 7/49 in controls to high dose). This increase was within the historical control range for dietary or oral gavage toxicity studies performed contemporaneously in this laboratory (Historical control range: 2-9 parafollicular cell adenomas) and therefore was not considered treatment related. No increase was noted in males.

Adrenals

There was a slight increase in the incidence of benign pheochromocytoma in males at 1000 mg/kg bw/day (4/50, 2/20, 2/24 and 9/50 in the controls to high dose). This increase was slightly higher than the historical control range (historical control range: 3-7), but there was no increased incidence observed in females and no increase in the incidence of the malignant form of the tumour. It should also be noted the incidence for control males in a study terminated in 2007 (within two years of this study) was 7/50, which is close to the incidence observed in the high dose males of this study. Hence, overall the incidence of benign pheochromocytoma is not considered treatment related.

Overall, in rats there were no neoplastic findings considered relevant to human health.

Mice

In the CD-1 mouse study, an increase in hepatocellular adenoma incidence was observed in males at all doses compared to the controls. There was also an increase in the incidence of carcinomas observed in top dose males.

The increased incidence in hepatocellular adenomas in males was not statistically significant nor dose-related, but did slightly exceed the laboratory historical control (2-9; Dec 2001-May 2004 and 4-12; 2007-2012) in both the low dose (13/50 - 26%) and high dose (14/50 – 28%) males, but not the mid dose group (9/50 – 18%). The incidence also slightly exceeded the historical control range in the Charles River historical control database (1.4-20%; from studies initiated between 2002 and 2006). The study also showed that many of the affected males had multiple hepatic tumours. Multiple tumours are relatively rare in this strain of mouse; however, as they were also noted in control animals they are not considered treatment related, but do suggest these animals were

particularly susceptible to developing liver tumours. No increase in the incidence of adenomas in female mice was observed in this study (hepatocellular incidence of adenomas: 3 (control), 1 (low dose), 0 (mid dose), 1 (high dose)).

A slight increase in the incidence of hepatocellular carcinoma was noted at the limit dose in males (4/50 (8%) compared to 1/50 (2%) in the contemporary control). No carcinomas were noted in females. The increase in male carcinoma incidence at the top dose was higher than either of the laboratory historical control ranges (0/50 - 2/50 (0-4%)), but was within the historical control range available for Charles River Labs where the incidence of hepatocellular hypertrophy from 13 studies conducted between 2002 and 2006 ranged from 0-15% (including an incidence of 6/60(10%) and 9/60(15%) in two 78 week studies). See tables 18a and 18b for further information on historical controls.

Table 18a. In-House Historical Control Values: Primary Hepatocellular Neoplasms in Male CD-1 Mice from 18-Month Dietary Oncogenicity Studies

	Study						
Organ/Observation	A	B	C	D	E	F	G
Necropsy Date:	12/2001	05/2003	12/2003	04-05/2004	9/2006	12/2009	9-10/2011
Final Report:	2002	2003	2005	2004	2007	2010	2012
Liver (number examined)	50	50	50	50	50	50	50
Number of animals with one or more adenoma	8	2	5	8	9	12	4
Number of animals with one or more carcinomas	3	1	0	1	1	2	0
Total Mice with Adenoma and/or Carcinoma	10	3	5	9	10	13	4

Table 18b. HCD data from Charles River (published March 2010, with 13 studies initiated between 2002 and 2006): Incidence of hepatocellular adenomas and carcinomas

	1	2	**3**	**4**	**5**	**6**	**7**	**8**	9	10	11	12	**13**	***Range***
Date	2002	2003	**2003**	2003	**2004**	2004	2004	2004	2005	2005	2005	2005	2006	
No. on study	110	50	**60**	60	**60**	60	50	60	75	60	70	50	60	
No. surviving to termination	NA	NA	**41**	48	**46**	42	40	51	49	16	18	16	49	
% Survival	NA	NA	**68.3**	80.0	**76.67**	70.0	80.0	85.0	65.3	26.67	25.71	32.0	81.67	
Study Duration (weeks)	104	104	**78**	78	**78**	78	78	78	96	104	104	104	78	
Hepatocellular Adenoma	5	10	**5**	4	**12**	6	3	5	10	1	1	4	5	***1-12 (20%)***
Hepatocellular Carcinoma	5	2	**6**	1	**9**	1	1	3	2	1			1	***1-9 (15%)***

4.10.1.2 Carcinogenicity: inhalation

No information available

4.10.1.3 Carcinogenicity: dermal

No information available

4.10.2 Human information

No information available

4.10.3 Other relevant information

Not applicable.

4.10.4 Summary and discussion of carcinogenicity

The carcinogenicity of pyroxsulam has been investigated in rats (Fischer 344) and mice (CD-1).

No treatment-related carcinogenic effects were observed in rats.

In the mouse, the incidence of liver adenomas and carcinomas was slightly higher than the contemporaneous and laboratory historical control in males, but the carcinomas were within the control range provided for Charles River Labs. In addition, whilst the incidence of adenomas was increased at the low and top dose groups, it was within the laboratory historical control range in the mid dose group. Further, these findings occurred in male mice only, which appeared to be susceptible to liver tumour formation with multiple adenomas (rather than single incidences) observed in the livers of both control and treated animals. In conclusion, it is considered that there is insufficient evidence in this study to conclude a treatment-related carcinogenic effect of pyroxsulam.

4.10.5 Comparison with criteria

As there is insufficient evidence for a carcinogenic effect in rats and mice, and there are no other concerns about the potential carcinogenicity of pyroxsulam, no classification is proposed.

4.10.6 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The chronic toxicity and carcinogenicity of pyroxsulam was investigated in two guideline and GLP compliant studies; one in the rat (Stebbins & Brooks, 2005 – original study report, 2008 – revised study report; which used F344 rats dosed with up to 1000 mg/kg bw/day), and one study in the mouse (Johnson *et al*., 2005; which used CD-1 mice dosed with up to 1000 mg/kg

bw/day). There were no treatment related adverse effects on mortality, clinical signs, ophthalmology, haematology, clinical chemistry, or histopathology. Some evidence was noted for slightly increased tumour incidences in both rats and mice.

Rats: Male rats given 100 or 1000 mg/kg bw/day had slightly higher incidences of large granular lymphocyte (LGL) leukaemia than controls (tabulated below). This tumour type may also be known as mononuclear cell leukaemia. These were considered by the DS not to be substance related. In males, mortality was slightly higher (but not statistically significant), during the last 5 weeks of the study at 100 and 1000 mg/kg bw/day and this may have been associated with the increase in leukaemia observed at that time. The mechanism of action for these tumours is unknown. There was no evidence for an early onset of LGL leukaemia in rats exposed to pyroxsulam.

Mice: In the 18-month dietary CD-1 mouse study, an increase in hepatocellular adenoma incidence was observed in males at all doses compared to the controls (tabulated below). There was also an increase in the incidence of carcinomas observed in top dose males. There was no substance related effect on mortality. Liver tumours arose late in the study. The mechanism of action for these tumours is unknown.

The DS has summarised the key data for both long-term studies in table 18 of the CLH report. The DS did not propose a carcinogenicity classification for pyroxsulam.

Neoplastic changes in rats

LGL leukaemia

This effect was specific to male F344 rats. Male F344 rats have a propensity to develop LGL leukaemia spontaneously with a variable and potentially high incidence. The reporting of historical control data was particularly useful in this case.

The incidence of LGL leukaemia in **males** given 100 or 1000 mg/kg bw/day was outside the historical control range (22-36)% of dietary or oral gavage toxicity studies (five in total) performed within a short time frame of the original study from 2005 (2002-2005) at the testing laboratory (table below).

The inbred F344/N rat sub-strain has also been used for US NTP rodent toxicity and carcinogenicity bioassays for more than thirty five years. According to the May 2009 NTP Historical Controls report (http://ntp.niehs.nih.gov; all routes/all vehicles, accessed 16-01-2016), the average incidence rate for leukaemia in males was 38.3% (536/1398; range 8–58%) and 21.3% in females (288/1350; range 8–40%). The NTP 2009 report may be considered the most relevant in this case because it was compiled from the most recent 5-year period of results. Studies conducted prior to 1995 by the NTP had a wider LGL leukaemia incidence range from 32 to 74% (mean 51%) for males. The more recent NTP bioassays were conducted with the NTP-2000 diet while the older studies used the NIH-07 diet. Briefly, the NTP-2000 diet is lower in protein, and higher in fat and fibre but with a similar calorific value. Some reports suggest being mindful of the diet used in a study because this may be a potential source of variability beyond that attributable to animal strain (Haseman *et al*., 2003).

The results for LGL leukaemia are within the historical control ranges for F344 rats used in the US NTP rodent toxicity and carcinogenicity bioassays regardless of the diet employed.

The incidence of LGL leukaemia in the 100 and 1000 mg/kg bw/day dose groups was similar even though the high dose group was ten times that of the mid dose group. This lack of a dose-related increase in tumour incidence raises doubt that the tumours are treatment related.

Neoplasms (Male rats, F344) in a 2-Year Feed Study of pyroxsulam. Dose mg/kg bw/day.

Tumour	HCD*	0	10	100	1000
Haematopoietic/Lymphoid System:					
Leukaemia, large granular lymphocyte (LGL)	77/255	20/50	21/50	28/50	29/50
Overall incidence	(30%)	(40%)	(42%)	(56%)	(58%)
Incidence range	22-36%				
Liver:					
Hepatocellular Adenoma	?	1/50	3/50	3/50	4/50
Overall incidence	(?%)	(2%)	(6%)	(6%)	(8%)
Incidence range	2-12%				
Adrenal:					
Pheochromocytoma, benign	?	4/50	2/20	2/24	9/50
Overall incidence	(?%)	(8%)	(10%)	(8%)	(18%)
Incidence range	6-14%				
Adrenal:					
Pheochromocytoma, malignant	12/846	1/50	1/20	0	0
Overall incidence	(1.4%)	(2%)	(5%)		
Incidence range	0-4%				

HCD is historical control data where known, with incidence mean (parenthesis) and incidence range from 5 studies conducted at the same testing laboratory with start dates of 2002-2005, for rats of the same strain and same supplier.

* Historical data taken from Pyroxsulam plant protection DAR (2012) and confirmed from the original study report.

? data not available or unknown.

Neoplasms (Female rats, F344) in a 2-Year Feed Study of pyroxsulam. Dose mg/kg bw/day.

Tumour	HCD*	0	10	100	1000
Haematopoietic/Lymphoid System:					
Leukaemia, large granular lymphocyte (LGL)	46/255	12/50	6/50	8/50	11/50
Overall incidence	(18%)	(24%)	(12%)	(16%)	(22%)
	12-24%				
Thyroid Gland:					
Adenoma, parafollicular cell	?	2/50	2/10	2/12	7/49
Overall incidence	(?%)	(4%)	(20%)	(17%)	(14%)
	4-18%				

HCD is historical control data where known, with incidence mean (parenthesis) and incidence range from 5 studies conducted at the same testing laboratory with start dates of 2002-2005, for rats of the same strain and same supplier.

* Historical data taken from Pyroxsulam plant protection DAR (2012) and confirmed from the original study report.

? Data not available or unknown.

An extensive investigation of the histopathology of several organs was carried out. Subsequent to finalisation of the initial study report, a review was conducted by industry on animals from the oncogenicity part of the study and this consisted of microscopic examination of all tissues and organs from five male and five female control animals, five male and five female high dose animals, and the spleens of all (50 animals/dose) male control and treated animals. The additional data was provided in order to confirm the incidence of LGL leukaemia in males at the low and mid dose. There was no evidence of any target effect on lymphoid tissues/organs (Lymph nodes / spleen / thymus). No substance-related increase in white blood cell count or

substance-related changes in differential white blood count in male rats was observed.

Liver Adenomas

Males exposed to 1000 mg/kg bw/day had a slightly increased incidence of hepatocellular adenomas (1/50, 3/50, 3/50 and 4/50 from controls to high dose). This increase was within the historical control ranges for dietary or oral gavage toxicity studies performed recently at the same testing laboratory and was therefore not considered treatment related. No increase was noted in females.

Thyroid Adenomas

Females given 1000 mg/kg bw/day had an increased incidence of parafollicular cell adenomas (2/50, 2/10, 2/12 and 7/49 from controls to high dose). This increase was also within historical control ranges of dietary or oral gavage toxicity studies performed recently at the testing laboratory and therefore was not considered treatment related. No dose response relationship was observed. A high spontaneous incidence was observed in a low number of animals for both the low and mid dose groups. No increase was noted in males.

Adrenal pheochromocytoma

Males exposed to 1000 mg/kg bw/day had an increased incidence of benign pheochromocytoma of the adrenal medulla (4/50, 2/20, 2/24 and 9/50 from controls to high dose). This was outside the historical control ranges for dietary or oral gavage toxicity studies performed recently at the same testing laboratory (6-14%). In addition, there was no increased incidence observed in females and no increase in the incidence of the malignant form of the tumour. According to the May 2009 NTP Historical Controls report (http://ntp.niehs.nih.gov; all routes/all vehicles, accessed 16-01-2016), the average incidence rate for pheochromocytoma in males was 14% (197/1395; range 6–22%), which illustrates the highly variable spontaneous incidence of this tumour. The incidence of pheochromocytoma does not exceed that seen in the NTP historical control data.

Summary

Increases in tumour incidences in male or female F344 rats exposed to pyroxsulam are not attributed to treatment but instead to high spontaneous and variable background levels in this particular strain. The DS concludes that in rats there were no neoplastic findings considered relevant to human health.

Neoplastic changes in mice

No substance related adverse effects were seen in females. The effects seen in males were increased liver weight, increased incidence of foci of altered hepatocytes and increased incidence and number of hepatocellular adenoma and carcinoma at the high dose.

The incidence of hepatocellular adenomas in males at all dose levels was outside concurrent controls (5/50, 13/50, 9/50, and 14/50 for controls to high dose) but there is no convincing dose response. Animals receiving 10 and 1000 mg/kg bw/day exceeded the in-house historical control range of 4-24% (table below). Studies conducted from 2002 to 2006 (available from the 2010 Charles River Laboratories report on spontaneous neoplasms in the CD-1 mouse) showed hepatocellular tumours incidences ranged from 2-20% for adenomas and 0-15% for carcinomas in males. This report confirms the variable and potentially high background incidence of hepatocellular tumours in male CD-1 mice.

A slight increase in the incidence of hepatocellular carcinoma was noted at the limit dose in males (4/50 compared to 1/50 in the contemporary control). No carcinomas were noted in

females. The increase in male carcinoma incidence at the top dose was higher than the laboratory historical control range (0/50 - 3/50), but was well within the historical control range available for Charles River Laboratories (0-15%).

There was no effect on hepatocellular adenoma incidence in females (3/50, 1/50, 0/50, 1/50, control group to high dose group). There was no observed genotoxicity; including an *in vivo* CD-1 mouse liver UDS study. The increased incidence in hepatocellular adenomas in males was not statistically significant.

Neoplasms (Male mice, CD1) in an 18-month Feed Study of pyroxsulam. Dose mg/kg bw/day.

Tumour	HCD	0	10	100	1000
Liver: Hepatocellular Adenoma Overall incidence	48/350 (14%) 4-24%	5/50 (10%)	13/50 (26%)	9/50 (18%)	14/50 (28%)
Liver: Hepatocellular Carcinoma Overall incidence	8/350 (2%) 0-6%	1/50 (2%)	0	2/50 (4%)	4/50 (8%)

HCD is the in-house historical control data range available from seven 18-month dietary oncogenicity studies which used the CD-1 mouse. The incidence of hepatocellular tumours from 7 studies with necropsy dates between 2001 and 2011 ranged from 4-24% for adenomas and 0-6% for carcinomas in males.

The lack of a dose response relationship in tumour incidence weakens the argument for a substance related effect at the high dose. The incidence of mice with adenomas was similar at 10 and 1000 mg/kg bw /day, but the systemic exposure to pyroxsulam, as indicated by the area under the curve (AUC), was 22-30 times higher in plasma, RBCs and liver at 1000 mg/kg bw/day (table below, adapted from table B.6.9 in the pyroxsulam DAR). This suggests there was no substance-related increase at 1000 mg/kg bw/day.

Plasma, RBC and liver kinetic data from ^{14}C-pyroxsulam in male mice

	Plasma			RBC			Liver		
PK parameters	10 mg/kg	100 mg/kg	1000 mg/kg	10 mg/kg	100 mg/kg	1000 mg/kg	10 mg/kg	100 mg/kg	1000 mg/kg
T_{max} (h)	0.5	1.0	2.0	0.5	1.0	1.0	0.5	1.0	4.0
C_{max} (µg g^{-1})	36.6	174.4	258.2	5	35.7	894.6	31.48	182.2	336.4
$AUC_{0\rightarrow t}$ (µg h g^{-1})	118.6	676.2	2562.7	20.4	128.3	610.8	139.2	783.0	3083.7

T_{max} - Time of maximum concentration
C_{max} - Maximum concentration
$AUC_{0\rightarrow t}$ - Area under the curve

The pattern of incidence of multiple adenomas was also similar at 10 and 1000 mg/kg bw/day (table below, adapted from Table B.6.48 in the pyroxsulam DAR), giving further support that there was no substance related effect. While multiple hepatic tumors were particularly common in males given 1000 mg/kg bw/day, they were also noted at all other dose levels including controls where one animal had six hepatic adenomas.

Number of male mice with multiple hepatocellular tumours following a lifetime exposure to pyroxsulam. Dose mg/kg bw/day

Observations		0	10	100	1000
Liver: primary adenomas;	incidence of 1	3	7	7	7
	incidence of 2	1	5	1	1

	incidence of 3	0	0	1	5
	incidence of 4	0	0	0	1
	incidence of 5	0	1	0	0
	incidence of 6	1	0	0	0
Liver:					
primary carcinomas;	incidence of 1	1	0	1	4
	incidence of 2	0	0	1	0

HCD: out of 4 studies conducted 2001-2004, 2 studies had 1 male with 2 adenomas, and 1 study had 1 male with 1 adenoma and 1 carcinoma.

Male mice also show an increase in pre-neoplastic lesions in the liver (altered foci) at 1000 mg/kg bw/day (table below). This finding was uncommon in control mice at the test laboratory. Of note however, there was no increase in basophilic foci, which is more commonly linked to tumour formation than other types of cell foci. The incidence of foci of altered cells in the liver of females from all dose levels was low and similar to controls.

Hepatocellular effects in male CD 1 mice following 18 months exposure to pyroxsulam. Dose mg/kg bw/day

Non-neoplastic findings	HCD	0	10	100	1000
Basophilic cell foci	0-1	1	2	1	2
Clear cell foci	0	0	0	0	7
Eosinophilic cell foci	0-3	0	0	1	3
Mixed cell foci	0-1	2	1	0	5
Total number of mice with a focus of altered cells		2	3	1	12
Focus of altered cells and primary tumour		1	0	1	7

HCD is the in-house historical control data from 4 studies necropsied 2001-May 2007 (50 males per control).

Summary

Increases in hepatic tumour incidences in male CD-1 mice exposed to pyroxsulam are not strongly associated with treatment and occur against high spontaneous and variable background levels in this particular strain. The DS concluded that there was insufficient evidence in this study to determine a treatment-related carcinogenic effect caused by pyroxsulam. The DS did not propose classification for carcinogenicity.

Comments received during public consultation

Two MSCA commented on carcinogenicity. One MSCA suggested that classification as Carc. 2; H351 should be considered. Another MSCA supported the proposal of the DS for no classification.

Additional key elements

Mouse historical control data from Charles River Laboratories (2010): This compilation reported incidences of neoplasms in Crl:CDl (ICR) mice, maintained as control animals, in studies of 78-104 weeks duration. The 14 studies included in this publication were initiated between 2002 and 2006 in five different laboratories. All studies used male and female Cri:CDI (ICR) mice from Charles River Laboratories production sites.

Summary of the mouse HCD for hepatocellular tumours in both males and females.

Incidence of hepatocellular tumours by study in CD-1 Mice: Charles River Labs report (2010) on spontaneous neoplasms in studies from 2002 - 2006

Male CD-1 Mice

Study Identification	1	2	3	4	5	6	7	8	9	10	11	12	13
LIVER	110	50	60	60	60	60	50	60	75	60	70	50	60
Hepatocellular Adenoma	5	10	5	4	12	6	3	5	10	1	1	4	5
Hepatocellular Carcinoma	5	2	6	1	9	1	1	3	2	1			1

Female CD-1 Mice

Study Identification	1	2	3	4	5	6	7	8	9	10	11	12	13	14
LIVER	110	47	60	60	60	60	50	60	75	60	70	50	60	75
Hepatocellular Adenoma		1								1	1			
Hepatocellular Carcinoma								1			1			

Assessment and comparison with the classification criteria

Relevance of LGL leukaemia in rats – a substance related effect?

The relevance of LGL leukaemia, also known as mononuclear cell leukaemia (MNCL) in the F344 rat for humans is questionable. A review by Caldwell (1999) is available, questioning the relevance of LGL induction by chemicals. LGL leukaemia is found at high incidence in untreated, aged F344 rats, which once was the standard strain for studies of the National Toxicology Program (NTP). The mechanism for the induction of LGL leukaemia in the F344 rat is unknown and substance induced incidences of this type of leukaemia in this strain of rat are generally not considered relevant for human hazard assessment by the Dutch RIVM (Muller, 2005).

The LGL leukaemia cells originate from the population of large granular lymphocytes. Large granular lymphocytes are cytotoxic T lymphocytes and natural killer cells which kill virally infected and tumorigenic cells by secreting cytotoxic proteins from their complement of intracellular granules onto their target cells. The tumour cells are always found in the spleen, often in the liver and sometimes in other organs. Hence the revision of the Stebbins & Brooks (2005) report in 2008 to investigate all spleens from all treated animals and to clarify the LGL response.

The incidences of leukaemia in male rats given 100 or 1000 mg/kg/day were outside of the limited in-house historical control data available (but not outside the HCD from the NTP). However, there are a number of factors that need to be considered which indicate that this effect is confounded by the high background incidence of this tumour type in F344 rats and is therefore not test substance-related (see also Gopinath, 2008):

1. The incidences were not statistically significant and showed no dose-relationship in spite of a 10-fold increase in dose between 100 and 1000 mg/kg/day.
2. Males were more susceptible than females. The results for females were comparable to or lower than the controls. There was no increase in LGL leukaemia in female rats.
3. Historical control data from the NTP (2009) report confirm the greater susceptibility of male F344 rats to spontaneous and variable development of LGL leukaemia relative to females.
4. There was no increase in LGL leukaemia in male or female mice.

5. There was no substance-related increase in white blood cell count or substance-related change in differential white blood cell count in male rats, which is consistent with an absence of a substance-related increase in LGL leukaemia.
6. The incidences of LGL leukaemia at 100 and 1000 mg/kg/day were well within the historical control range of the studies from the US NTP database.
7. Toxicokinetic data confirmed systemic and tissue exposures but this did not correlate with the increased incidence of leukaemia reported in male rats from treated groups (48h after a single dose of 1000 mg/kg bw, levels of pyroxsulam equivalents in plasma, RBCs, liver and spleen were 58-71x the levels at 10 mg/kg bw). There were 21/50 animals effected at the low dose level and 28/50 animals effected at the mid dose level compared to 29/50 in the high dose group - a 100-fold increase in dietary exposure. Therefore, there was no dose-related increase in LGL leukaemia in rats exposed to pyroxsulam.
8. There was no evidence for an early onset of LGL leukaemia in male rats.
9. An extensive evaluation revealed there was no evidence of any target effect on lymphoid tissues/organs (Lymph nodes / spleen / thymus).
10. Pyroxsulam is not genotoxic in *in vitro* and *in vivo* assays.
11. A close, structural, triazolopyrimidine analogue, penoxsulam, also showed a non-dose related increase in the incidence of LGL leukaemia in male rats from all dose groups when compared to the controls but this effect was also concluded by an expert panel to be not test substance-related, a decision supported by EFSA and the final EU review of this particular substance.

In agreement with the DS, the RAC concludes there is insufficient evidence for a treatment related carcinogenic effect of pyroxsulam in male F344 rats.

Relevance of Hepatocellular Tumours in CD-1 Mice

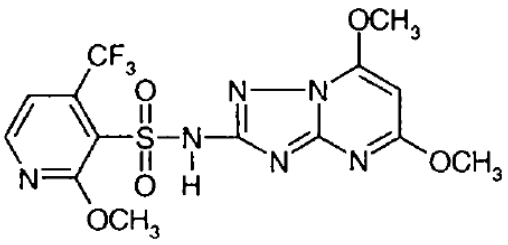
The effects seen after the chronic exposure of mice to pyroxsulam were hepatocellular foci of change (table 10) and a small increase in adenomas (5/50, 13/50, 9/50, and 14/50 for controls to high dose) and carcinomas (1/50, 0/50, 2/50, and 4/50 for controls to high dose) observed at 18 months (late onset) in the high dose males. The results from the 18-month mouse study are as follows:

1. Liver hepatocellular tumors were observed in the CD-1 mouse. This strain of mouse has a variable and often high background incidence of this tumour type (table below).
2. The increase in tumours was specific to **males only**. This effect is also confirmed by the historical control data from Charles River Laboratories (table below).
3. Adenomas were observed at all dose levels and at incidences greater than the concurrent controls. Incidences at the low and high dose were just outside the ranges for HCD. There was no dose response relationship.
4. Carcinomas were increased in the mid and high dose groups. A dose response relationship is questionable based on the incidence of carcinomas in the HCD. There is a high background of carcinomas in the CRL CD-1 mouse studies (0-15%).
5. The liver tumours were observed in CD-1 mice only and not in F344 rats.

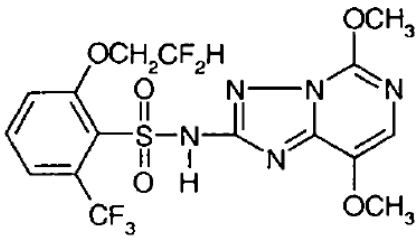
6. There was no multisite response, only the liver was involved.
7. There was no evidence of reduced tumour latency.
8. The structurally similar substance penoxsulam did not show a treatment-related increase in hepatocellular adenomas or carcinomas using the same strain of mouse. Penoxsulam did however confirm the male specific tumorigenic response, and the variable and high background incidence of tumours in the CD-1 mouse.

Penoxsulam 18-month CD-1 mouse carcinogenicity study results for liver tumours

Liver neoplastic findings for DE-638	**Males**				Females			
Dosage (mg/kg bw/day); $n = 50$	**0**	**10**	**100**	**375**	0	10	100	750
No of mice with adenomas	**8**	**9**	**7**	**3**	1	0	1	2
No. of mice with carcinomas	3	1	4	0	0	0	0	0
No. of mice with adenomas and/or carcinomas	**10**	**10**	**10**	**3**	0	0	0	0



Pyroxsulam



Penoxsulam

9. There was no confounding of results with excessive toxicity.
10. The mode of action and mechanism of action are unknown. There was no mechanistic data available.
11. The mouse metabolism study indicated a dose-response relationship in systemic exposure, but there was no clear dose-response relationship in the incidence of tumours.
12. There was no increase in basophilic foci.
13. There was no statistically significant trend in tumour incidence.
14. There was no impact on survival indices.
15. There was no evidence of genotoxicity; including an *in vivo* CD-1 mouse liver UDS study.

The high incidence of adenomas in the 10 and 1000 mg/kg bw/day dose groups just slightly exceeds the available historical control data. The observed effects are borderline and are not sufficiently above background to conclude that there is a treatment-related response.

The difference in response to pyroxsulam between males and females is striking. There were no liver effects in females given up to 1000 mg/kg/day that were attributed to treatment. The mean liver weights of females from all dose levels were almost identical to controls and the incidence of both foci of altered cells and hepatocellular adenomas was low and similar to controls. No carcinomas were noted in females.

The only apparent dose response is that shown by the incidence in hepatocellular carcinomas

(1/50, 0/50, 2/50, and 4/50) and supported by an increase in some presumptive pre-neoplastic lesions in the liver (altered foci) at 1000 mg/kg bw/day. However, four key pieces of information cast doubt on whether a real substance-related response is observed: (1) the HCD for CD-1 males confirms a variable and high background level of carcinomas in CD-1 mice, (2) this is confirmed with results from a close structural analogue (penoxsulam), (3) there is no dose response relationship with the increase in basophilic cell foci, and (4) the systemic exposure to pyroxsulam (as indicated by the AUC) was 22-30 times higher in plasma, RBCs and liver at 1000 mg/kg bw/day compared with the low dose group. This suggests there was no substance-related increase because the tumour profile was similar between the low and high dose groups.

The UK (RMS for the plant protection product DAR, 2012) considered there was a possible substance-related increase in the hepatocellular tumours at the top dose in male mice but this was not sufficient to warrant classification. The US EPA (2007) concluded the tumours in the low- and high-dose groups were unrelated to treatment due to the highly variable background levels of liver tumors in the male CD-1 mouse. The EFSA (2013) conclusion on pyroxsulam considered the liver tumours in mice did not support classification for carcinogenicity.

In agreement with the DS, RAC concludes that there is insufficient evidence for a substance related carcinogenic effect by pyroxsulam in male CD-1 mice. Females are unaffected. Effects seen at the high dose are confounded by a clear absence of a dose response relationship and a variable and high background level of hepatocellular tumours.

The evidence for substance-related carcinogenicity in Animals is equivocal

1. There is no causal relationship established between pyroxsulam dose and an increased incidence of benign and malignant tumours in rodents.
2. There is an increase in the incidence of tumours in two species but in one sex only . The tumour type in both species is common and variable. The incidences of mouse liver tumours just barely exceed historical control levels but the magnitude of the tumour incidence is unconvincing with regard to determining a true substance-mediated effect.
3. The malignant neoplasms in mouse liver at the highest dose of pyroxsulam do not exceed the HCD from CRL.

Comparison with the Criteria

There is no epidemiological evidence regarding the carcinogenicity of pyroxsulam in humans which would indicate Category 1A..

A substance shall be classified as *carcinogenic in category 1B* if: 'It is presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.' This category depends on strength of evidence, which consists of animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity. This means a causal relationship has been established between the chemical agent and an increased incidence of malignant neoplasms *or* of an appropriate combination of benign and malignant neoplasms in:

(a) two or more species of animals *or* in two or more independent studies in one species carried out at different times (or in different laboratories or under different protocols);

(b) both sexes of a single species;

(c) occurrence of malignant neoplasm to an unusual degree with regard to the

incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.

These criteria are not met with the substance pyroxsulam. Effects are confined to one sex and occur against a high background. Classification as category 1B is therefore not supported, since there is no firm evidence from animal experiments to demonstrate a strong substance related effect.

According to the CLP regulation a substance shall be classified as *carcinogenic in category 2* if: 'It is a suspected human carcinogen, but the evidence is not sufficient for category 1A or 1B'. Classification with Carc. 2 is justified if the following considerations are true:

(a) The evidence is limited to a single experiment: **Equivocal** - there was an increase in hepatocellular tumours in mice with an unclear relationship to dose;

(b) There are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies: **Not applicable** - Studies were conducted to guidelines and GLP;

(c) The agent increases the incidence only of benign neoplasm or lesions of uncertain neoplastic potential: **Equivocal;** *or*

(d) The evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs: **Unclear whether or not this is the case**.

(e) There are several other points to note that decrease the levels of concern for the observed rodent tumours and thus support no classification with category 2. These are outlined under sections (1) and (2) above.

Classification as category 2 is not supported by RAC. The occurrence of LGL leukaemia in the F344 rat is not considered to be substance related and is therefore not considered to warrant classification of pyroxsulam for carcinogenicity. The occurrence of hepatocellular tumours in CD-1 mice is regarded as very weak evidence for a possible substance related effect. The evidence is not strong enough to suggest a connection between dose and effect. The tumours are common and variable in incidence in CD-1 mice and it is not possible to conclude if pyroxsulam has an effect on the occurrence of hepatic tumours. Accordingly, **classification for carcinogenicity is not warranted** for pyroxsulam.

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

Table 19: Summary table of relevant reproductive toxicity studies - Fertility

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Two-generation study OECD 416 (2001) – deviation: no functional investigation of pups and no examination of reproductive tissues of weanlings GLP Oral(dietary) 27 Sprague-Dawley rats/ sex/dose Carney, Zablotny, Stebbins (2005)	Males P1 generation – 0, 106, 321 and 1078 mg/kg bw/day P2-generation – 112, 344 and 1138 mg/kg bw/day Females P1 generation – 104, 311, 1043 mg/kg bw/day P2 generation – 104, 316 and 1049 mg/kg bw/day	Parental toxicity Parental toxicity was limited to a very low incidence of perineal staining in the 300 mg/kg bw/day and 1000 mg/kg bw/day dose groups – this effect was not considered adverse. Erosion of the stomach was observed in P1 females treated with 300 mg/kg bw/day and 3 at 1000 mg/kg bw/day. The extent of the finding was very slight. The effects were not considered treatment related as not observed in the P2 animals. Reproductive toxicity No effects of treatment on mating, conception, fertility or gestation indices, post-implantation loss, time to mating, or gestation length in either generation Offspring effects 1000 mg/kg bw/day A small non-statistically significant decrease in pup weight in F1 males and females on day 21 (circa. 1 g) and F1 and F2 males on day 22 (circa 2-3g). When the individual pup weights considered, the difference did not appear biologically significant. A NOAEL for parental, reproductive and offspring effects of 1000 mg/kg bw/day was derived.

4.11.1.1 Non-human information

Information on reproductive toxicity is available from a 2-generation study in Sprague-Dawley rats.

In the study no adverse effects on reproductive toxicity was observed. The only effect on offspring observed was slightly reduced bodyweight. However, when the individual weights of the pups were considered, the difference did not appear to be biologically significant. Overall, the results suggest pyroxsulam does not affect fertility or reproductive performance.

4.11.1.2 Human information

No information available.

4.11.2 Developmental toxicity

Table 20: Summary table of relevant reproductive toxicity studies - Development

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Developmental toxicity OECD 414 (2001) – dosing started on day 6 GLP 26 female Sprague-Dawley rats/dose Oral gavage Sloter (2005a)	0, 100, 300 or 1000 mg/kg bw/day from day 6 to day 20 Vehicle – 0.5% methylcellulose	Maternal toxicity No maternal toxicity was observed Fetal examination The incidence of testicular alternations was slightly increased at 1000 mg/kg bw/day compared to controls (concurrent and historical). The observations were observed in three litters: • Missing testes (malformation) in one foetus from one litter; • Hypoplastic testis (malformation) in one foetus from another litter • Cystic testis (variation) in three fetuses from another litter In addition, one foetus from the 300 mg/kg bw/day also had a missing testis. A NOAEL for maternal and Foetal toxicity of 1000 mg/kg bw/day was derived.
Developmental toxicity study OECD 414 (2001) GLP Oral gavage 26 New Zealand White rabbits Sloter (2005b)	0, 30, 100 and 300 mg/kg bw/day in 0.5% methylcellulose – doses based on the results of a preliminary study, in which slight toxicity was observed at 300 mg/kg bw/day (decreased faecal output, mean body weight and food consumption)	Maternal toxicity One female from each treatment group died – these deaths were not considered treatment related. No treatment-related effects on bodyweight were observed at any dose level. Transient ↓ in food consumption at 300 mg/kg bw/day Foetal examination Absence of small gall bladder was observed in several foetuses from treated groups (3.5%, 2.9, 2.8% of fetuses per litter, low to high dose), but not in the controls. Since the % affected foetuses per litter only exceeded the historical control range (0.8%; 0-3%) at the lowest dose, the effect is not considered treatment related. Slight increases in the incidence of a few skeletal variations were noted, principally in the top dose group, but these were not statistically significant and were either well within the historical control range or showed no clear effect in terms of total foetal or litter incidence. No testicular effects were observed. A NOAEL of 300 mg/kg bw/day for maternal and foetal toxicity was derived.

4.11.2.1 Non-human information

Information is available from developmental toxicity studies in rat and rabbit.

In the rat study, conducted up to 1000 mg/kg bw/day, the only effects of concern were in the testes (see table for details). These effects were dismissed by the study authors on the basis of their low incidence and the fact that similar effects were not noted in the 2-generation study conducted at equivalent doses (although the 2-generation study was a dietary study whereas the developmental study was via oral gavage). In addition, the applicants also provided additional arguments to support their opinion why they were not substance related.

1) "...compounds known to produce hypoplastic testis and/or missing testis are androgen receptor antagonists or endocrine active agents (Foster and Harris, 2005; Carruthers and Foster, 2005; Anway et al, 2005). These compounds have been shown to interfere with the development of the male reproductive tract, embryonic testis development and male fertility. Male fertility in these particular studies was measured by different parameters (e.g. sperm mobility, sperm counts, testis and epididymal weights, anogenitial distance). Pyroxsulam had no effect on any of these parameters; this lack of correlation is another piece of the supportive data to consider these findings in the rat developmental study as not treatment related".

2) Reproductive alterations are rarely observed in isolation, but instead individual pups and multiple pups, within a litter, will have a suite of treatment-related effects (Foster and Harris, 2005, Carruthers ND Foster, 2005). In addition, for pyroxsulam, the absence of less serious effects that would be expected to precede the more serious effects of missing or hypoplastic testes was noted (reference to Bay, 2006). In particular, Rasoulpour proposes that cryptochrism, hypospadias and decreased testes and accessory organ weight and sperm counts would occur long before any treatment-induced missing or hypoplastic testes.

3) Testis cysts (clear sacs with a bubble like appearance) are incidental observations and not related to the hypoplastic or missing testes. This conclusion was based on data mining (no reference to similar testis cysts on normal or treated rats was found) and consultation with US and European test laboratories (most laboratories do not record these cysts). The consensus of the reproductive toxicologists consulted was that because the effect occurred in three pups from one litter it was an incidental finding.

Given the low incidence of the effects observed at the limit dose (1000 mg/kg bw/day), the absence of associated findings and the fact that similar findings were not observed in the 2-generation study or the rabbit developmental study, the effects are considered spontaneous and not treatment related. Overall, the results suggest that pyroxsulam does not cause developmental toxicity in rats.

In the rabbit developmental toxicity study, no significant maternal toxicity or evidence of treatment related effects were observed, suggesting pyroxsulam is not a developmental toxicant in rabbits.

4.11.2.2 Human information

No information available

4.11.3 Other relevant information

Not applicable

4.11.4 Summary and discussion of reproductive toxicity

Effects on fertility were investigated in a two-generation study in rats. In the study no adverse effects on reproductive toxicity was observed up to a dose of 1000 mg/kg bw/day. The only effect on offspring observed was slightly reduced bodyweight. However, when the individual weights of the pups were considered, the difference did not appear to be biologically significant. Overall, the results suggest pyroxsulam does not affect fertility or reproductive performance.

The developmental toxicity of pyroxsulam has been investigated in a developmental toxicity study in rats and rabbits. In rats, the only effects were testicular effects (missing testes, hypoplastic testes) in offspring. However, given the low incidence, the lack of related effects and failure to see similar

effects in the 2-generation study or the rabbit developmental study, these findings were not considered treatment related. In rabbits, no malformations of concern were observed. Overall, there was no evidence of a direct adverse effect on development.

4.11.5 Comparison with criteria

No effects were observed that provide sufficient evidence to cause a strong suspicion of impaired fertility or developmental toxicity.

4.11.6 Conclusions on classification and labelling

Not Classified: conclusive but not sufficient for classification

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

The effects of pyroxsulam on reproductive toxicity were investigated in a single OECD 416 (2001), 2-generation, GLP compliant study in Sprague-Dawley rats by Carney *et al*. (2005). Pyroxsulam was administered to 27 CD (CrlCD(SD) IGC BR) rats/sex/dose in the diet at nominal dose levels of 0, 100, 300, and 1000 mg/kg bw/day.

Parental animals

There were no adverse effects on parental survival, clinical signs, body weight/gain, and food consumption in either sex in either generation. Alterations in organ weights were not consistent between generations and/or did not demonstrate a dose-response relationship. There were no treatment-related alterations in gross necropsy or histopathology in either sex or generation. There were no effects on reproductive indices in either males or females.

Offspring

There were no effects of treatment at any dose level on gestation indices, post-implantation loss, pup survival, or pup sex ratio in either generation. The only parameter reported as significantly altered was F2 pup survival, which was significantly increased on PND 21 at the 300 mg/kg/day dose level. There was a slight, non-statistically significant reduction in F1 pup body weights at the highest dose on day 21 and similarly for male F1 and F2 pups at day 22 but these effects were not considered adverse or biologically significant.

Summary

Pyroxsulam was well tolerated by both sexes throughout the premating and mating periods at dose levels up to the limit dose (1000 mg/kg bw/day, highest dose tested). No adverse effects were observed in either generation on the reproductive function of either sex or on the survival, growth, and development of the offspring. The NOAEL for reproductive toxicity was 1000 mg/kg bw/day, based on the lack of any significant adverse effect on any parameter. Similarly, the NOAEL for parental and offspring toxicity is 1000 mg/kg bw/day, the highest dose tested.

The DS did not propose classification for fertility.

Development

The developmental toxicity of pyroxsulam was investigated in a developmental toxicity study in rats and rabbits.

Rat Developmental Study (Carney & Tornesi, 2005)

In an OECD 414 guideline, GLP compliant study, pyroxsulam was administered to 26 time-mated Crl:CD (SD) female rats/dose via gavage at dose levels of 0, 100, 300, or 1000 mg/kg bw/day from days 6 through 20 of gestation.

Administration of pyroxsulam via oral gavage at dose levels up to 1000 mg/kg bw/day produced no treatment related maternal toxicity. The occurrence of a number of visceral and skeletal variations were comparable among the groups and occurred at a very low incidence (one variation/foetus/dose), or did not show a dose-related increase with dose as evidenced by the occurrence of two malformed foetuses in the control, one in the 100 mg/kg bw/day group, nine in the 300 mg/kg bw/day group, and three in the 1000 mg/kg bw/day group. There were no statistically significant differences in the incidence of any foetal malformation or variation in any of the treated groups compared to the control. These were considered to be spontaneous changes rather than substance-related effects.

The only effects of concern were in the testes where the incidence of a number of testicular alterations was slightly increased at 1000 mg/kg bw/day compared with controls and with recent historical negative control data (table below).

Alterations to the foetal testes in the rat developmental toxicity study.

Fetal variation	Foetal or litter incidence	0 mg/kg bw/day	Historical negative control mean†	100 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day
Cyst testis (variation)	F	0/66 (0.0)	0.0	1/70 (1.4)	0/58 (0.0)	**3**/75 (4.0)
	L	0/22 (0.0)	0.0	1/24 (4.2)	0/20 (0.0)	**1**/21[x] (4.8)
Hypoplastic testis (malformation)	F	0/66 (0.0)	0.0	0/70 (0.0)	0/58 (0.0)	**1**/75 (1.3)
	L	0/22 (0.0)	0.0	0/24 (0.0)	0/20 (0.0)	**1**/21[y] (4.8)
Mising testis (malformation)	F	0/66 (0.0)	0.0	0/70 (0.0)	1/58 (1.7)	**1**/75 (1.3)
	L	0/22 (0.0)	0.0	0/24 (0.0)	1/20 (5.0)	**1**/21[z] (4.8)

† HCD: stated in the Pyroxsulam plant protection DAR (2012) - testing laboratory control data, same rat strain, same supplier, 5 studies 2004 – 2006.
x,y z: effect observed in independent litters; cystic testis was seen in 3 foetuses from the same litter (litter x); the two other foetuses with testicular alterations were each from different litters (y and z).

The testicular alterations noted were seen in three litters at **1000** mg/kg bw/day:

- missing testis (malformation) in one foetus from one litter,
- hypoplastic testis (malformation) in one foetus from another litter,
- cystic testis (variation) in three foetuses from another litter.

These findings in the rat developmental study with pyroxsulam are considered spontaneous and not substance-related for a number of reasons as outlined originally by the industry applicant:

1. These testicular findings are at an extremely low incidence and found in isolated litters. Reproductive system alterations are rarely observed in isolation, but instead individual pups, and multiple pups within a litter, will have a suite of treatment-related effects.
2. There were no effects on the testes noted in the rat 2-generation study or the rabbit developmental study.
3. Fertility indices of adult males and sperm parameters were unaffected in the rat 2-generation study or the rabbit developmental study.
4. The absence of less serious effects (cryptorchidism, hypospadias, and decreased testis and accessory organ weights and sperm count) in the multigeneration study at 1000 mg/kg bw/day was noted. These would be expected to precede the more serious effects of missing or hypoplastic testes.
5. Testicular cysts are incidental observations and not related to hypoplastic or missing testes.

Compounds known to produce hypoplastic testis and/or missing testis are androgen receptor antagonists or endocrine disruptors. Such compounds have been shown to interfere with the development of the male reproductive tract, embryonic testis development and male fertility. The anticipated effects on male fertility if pyroxsulam acted in a similar manner was not seen in the rat 2-generation study.

Pyroxsulam, at dose levels up to 1000 mg/kg bw/day produced no treatment related maternal toxicity. The occurrence at 1000 mg/kg bw/day of three litters with testicular alterations compared with a concurrent and historical control incidence of zero, (in the absence of maternal toxicity), is not substance-related.

<u>Rabbit Dose-Ranging Developmental Study (Sloter, 2005a)</u>

Pyroxsulam was administered orally by gavage to five groups of six time mated female New Zealand White rabbits once daily from gestation days 6 through 28. Dosage levels were 10, 100, 300, 600 and 1000 mg/kg bw/day.

There was significant and severe maternal toxicity (severely decreased body weights and food consumption, leading to early termination) at the highest dose of 1000 mg/kg bw/day. Based on the results of this study, dosage levels of 30, 100 and 300 mg/kg bw/day were selected for a definitive prenatal developmental toxicity study of pyroxsulam administered orally by gavage to pregnant rabbits.

<u>Rabbit Developmental Study (Sloter, 2005b)</u>

Pyroxsulam, was administered orally by gavage to groups of 26 time mated female New

Zealand White rabbits once daily from gestation days 6 through 28. Dosage levels were 0, 30, 100 and 300 mg/kg bw/day.

Absent or small gallbladder was seen in several foetuses from the treated groups but in none of the controls. There was however, no evidence of a dose-related increase in incidence and it was thus concluded that there was no substance-related effect. Slight increases in the incidence (mean % affected foetuses per litter) of a few skeletal variations were noted, principally in the top dose group, but these were not statistically significant and were either well within the historical control range or showed no clear effect in terms of total foetal or litter incidence. It is notable that compared to the findings of the rat developmental toxicity study, no testicular variations or malformations were noted in rabbit foetuses.

No definitive adverse signs of maternal toxicity, and no evidence of substance-related developmental toxicity were observed in this study.

The DS does not propose classification for developmental toxicity.

Comments received during public consultation

One MS agreed with the proposal of the DS for no classification for reproductive toxicity.

Assessment and comparison with the classification criteria

Fertility

In the rat 2-generation study, no adverse effects were observed in either generation on the reproductive function of either sex or on the survival, growth, and development of the offspring up to the limit dose of 1000 mg/kg bw/day.

The results show that pyroxsulam does not affect fertility or reproductive performance. There is no evidence to classify pryoxsulam for fertility effects.

RAC agrees with the DS, **classification for fertility is not warran**ted.

Developmental Toxicity

In the rat developmental study, conducted up to 1000 mg/kg bw/day, the only effects of concern were in the testes. Given the low incidence of the effects observed at the limit dose (1000 mg/kg bw/day), the absence of associated findings and the fact that similar findings were not observed in the 2-generation rat study or the rabbit developmental study, the effects were considered by the DS to be spontaneous and not treatment related.

RAC agrees with the conclusion of the DS, pyroxsulam does not cause developmental toxicity in rats or rabbits. Accordingly, **classification for developmental effects is not warranted**.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Method	Dose Levels	Observations and Remarks	Reference
Chronic neurotoxicity study (one-year) OECD 424 (1997) GLP Dietary exposure 10 Fischer 344 rats/sex/dose	0, 10, 100 and 1000 mg/kg bw/day	4.4% ↓ bodyweight (based on all animals in this combined chronic/carcinogenicity and neurotoxicity study) was slightly reduced in females. Increased perineal staining in females and limited evidence of this effect in males There were no substance related effects on FOB findings or motor activity. There were no macroscopic or microscopic effects observed in the central or peripheral nervous systems. A NOAEL of 1000 mg/kg bw/day was derived for neurotoxicity	Maurissen, Andrus, Yano and Brooks (2005)

In a one-year neurotoxicity study in rats, there were no neuropathological findings in the central and peripheral nervous systems or any effects in the functional observation battery or on motor activity suggestive of neurotoxicity. Overall, pyroxsulam does not appear to be neurotoxic.

4.12.1.2 Immunotoxicity

No information available

4.12.1.3 Specific investigations: other studies

Not applicable

4.12.1.4 Human information

4.12.2 Summary and discussion

No neurotoxic effects were observed in a one year neurotoxicity study in rats up to a dose of 1000 mg/kg bw/day (Maurissen *et al*, 2005).

4.12.3 Comparison with criteria

No neurotoxic effects were observed in a one year neurotoxicity study in rats up to a dose of 1000 mg/kg bw/day (Maurissen *et al*, 2005).

4.12.4 Conclusions on classification and labelling

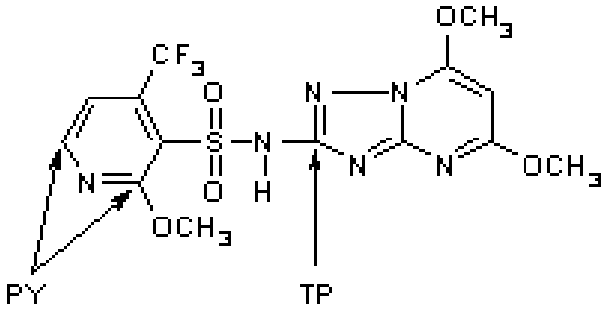
Not Classified: conclusive but not sufficient for classification

5 ENVIRONMENTAL HAZARD ASSESSMENT

Pyroxsulam (referred to in test reports as XDE-742) is a systemic post-emergence herbicide used for weed control. It is absorbed by foliage but also plant roots. Available environmental fate and hazard studies have been considered under Directive 91/414/EEC (subsequently replaced by EU Regulation 1107/2009) and summarised in the Draft Assessment Report, 2012 and subsequent DAR Addenda (Volume 3, B8; Environmental Fate and Behaviour and Volume 3, B9: Ecotoxicology). The agreed endpoints from the peer review of pyroxsulam under Directive 91/414/EEC are also included in the 2013 EFSA Conclusion.

The key information pertinent to determining a classification is presented below. All radiolabelled studies used ^{14}C-pyroxsulam with a purity of >97% and up to two labels as shown in Figure 1.

Figure 1: Structure of pyroxsulam indicating positions of the ^{14}C labels.



PY = 2- and 6- positions of pyridine ring
TP = 2-position of triazolopyrimidine ring

Pyroxsulam has a measured dissociation constant of 4.67 at 20^{o}C (Cathie, 2004). It is anticipated pyroxsulam will exist in its dissociated form at environmentally relevant pH (e.g. estimated 17.5% ionized at pH4, 68% ionised at pH 5, 95.5% ionised at pH 6, and 99.5% ionised at pH 7).

Where available information on degradation products is included – full details of degradant names and structures are presented in Annex I.

5.1 Degradation

A summary of available valid information on the fate of pyroxsulam is presented in Table 21 below.

Table 21: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Aquatic hydrolysis EPA Guideline (Subdivision N, 161-1) and SETAC Guideline (Part 1, section 9), GLP	Stable at pH 5, 7 and 9 at 20 ^{o}C	Valid	Yoder, 2004
Aquatic photolysis EPA (Subdivision N, 161-2) and SETAC Guideline (Part 1, section 10.1), GLP	Pyroxsulam DT_{50} = 4.1 days at 40^{o}N in summer sunlight. Degradant pyridine sulfinic acid DT_{50} = 32 days at 40^{o}N in summer sunlight. Degradant ADTP DT_{50} = 32 to 41 days at 40^{o}N in summer sunlight.	Valid	Byrne *et al*, 2006
Ready biodegradation OECD Guideline 301B, GLP	Not rapidly biodegradable 19-23% degradation after 28 days	Valid	Schwarz, 2003
Water/sediment simulation SETAC Guideline (Part 1, section 8.2) and BBA Guideline (Part IV, section 5-1), GLP	Dissipation DT_{50} days based on whole system: 12 to 24 days Degradation DT_{50} days based on whole system: 17 to 33 days Mineralisation: 0.8 to 2% AR at 101 days	Valid Aerobic system	Yoder *et al*, 2006c

5.1.1 Stability

Aqueous hydrolysis

An aqueous hydrolysis study (Yoder, 2004) is available following GLP, US EPA Guideline Subdivision N, Series 161-1 and SETAC Guideline part 1, section 9. The study used ^{14}C radio labelled pyroxsulam (0.1 mg a.s./l). Test solutions were incubated at 20 ^{o}C in the dark for 32 days. No significant degradation was observed and analysis showed 100% radioactivity as pyroxsulam at study termination. On this basis, pyroxsulam is considered hydrolytically stable.

Aqueous photolysis

An aqueous photolysis study (Byrne *et al*, 2006) is available following GLP, US EPA Guideline Subdivision N, Series 161-2 and SETAC Guideline part 1, section 10.1. The study used ^{14}C radio labelled pyroxsulam (1.0 mg a.s./l). Test solutions were incubated at pH 7 for 15 days at 20 ^{o}C under constant irradiation using a xenon lamp (wavelengths below 290 nm filtered out). This is considered equivalent to 73.5 days of non-continuous irradiation in summer sun at 40^{o}N.

The quantum yield of pyroxsulam was determined using an actinometer to be 4.41 x10^{-1}. This results in a predicted DT_{50} of 3.2 days at 40^{o}N in summer sunlight. Correcting for lamp intensity, the experimental DT_{50} for pyroxsulam at 40^{o}N in summer sunlight was 4.1 days.

Photodegradation is considered to occur through cleavage of the sulphonamide bridge resulting in the degradants pyridine sulfinic acid and ADTP. The DT_{50} of pyridine sulfinic acid was determined to be 32 days at 40°N in summer sunlight. The DT_{50} of ADTP was determined to be 32-41 days at 40°N in summer sunlight.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Not available

5.1.2.2 Screening tests

A ready biodegradation study (Schwarz, 2003) is available following OECD Guideline 301B (CO_2 Evolution) and GLP using pyroxsulam. Activated sludge from a laboratory wastewater plant treating municipal sewage was used at 30 mg/l with 52 mg test item. Validation criteria for the Reference and Toxicity Controls were met. Ultimate biodegradation reached a maximum of 19 and 23% in the duplicate samples over 28 days. Overall, the substance is considered not readily biodegradable.

5.1.2.3 Simulation tests

A distribution and degradation in aerobic water-sediment system study (Yoder *et al*, 2006c) is available following SETAC Guideline (Part 1, section 8.2) and BBA Guideline (Part IV, section 5-1). The study used ^{14}C-pyroxsulam with two labels. Two aerobic systems were used: 'UK' and 'France'. The water and sediment test conditions are included in Table 22 below. The system was treated with 0.016 mg pyroxsulam per litre of water via the water surface.

Table 22: Water-sediment system test conditions

Criteria	River Roding, UK	Haut Languedoc, France
Water properties	pH: 8.3 Dissolved organic carbon: 4.8 ppm Oxygen: 0.2 mg/l at start to 2.1 mg/l at end Redox potential: 125.9 mV at start to 22.4 mV at end	pH: 8.1 Dissolved organic carbon: 5.8 ppm Oxygen: 5.0 mg/l at start to 2.0 mg/l at end Redox potential: 228.4 mV at start to 20.8 mV at end
Sediment properties	46% sand; 26% silt; 28% clay Organic carbon 2.2% at start pH: 7.3 Redox potential: -177.3mV at start to -149.2 mV at end	88% sand; 10% silt; 2% clay Organic carbon 2.9% at start pH: 4.8 Redox potential: -74.1 mV at start to -72.0 mV at end

The study was conducted at 20 °C, in the dark under aerobic conditions for up to 101 days.

Radioactivity was determined by Liquid Scintillation Counting (LSC) and subsequent analysis by High Performance Liquid Chromatography (HPLC) was undertaken. Total mean recoveries for both systems were >90% Applied Radioactivity (AR) for both labels at each sampling point.

Pyroxsulam dissipated from the water phase to the sediment phase in both systems where degradation to 7-OH-XDE-742 and ATSA occurred (Figure 2 shows the proposed aerobic degradation pathway). A third degradant was observed at >10% AR (max. 16.5% AR) but was unable to be identified. Further investigation was unable to recreate the compound and it was concluded that it was an experimental anomaly.

In water pyroxsulam decreased from initial 84-103.4% AR to 14.4-22.1% AR on day 101. In sediment pyroxsulam increased from initial 0.8–16.1% AR to peak between 17.2 and 42% AR by day 75.

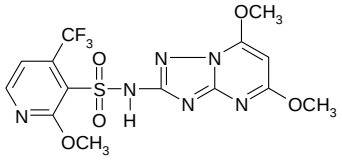
A Single First Order (SFO) kinetics approach was applied to calculated DT_{50} values. The study authors removed outliers and refitted the model to improve fit. While this approach was not statistically justified, overall slower rates were derived and the approach was accepted in the DAR.

Whole system dissipation DT_{50} values for both labels were as follows:

Pyroxsulam $DT_{50\ whole\ system}$: 24 days for UK system and 12 days for France system
7-OH-XDE-472 $DT_{50\ whole\ system}$: 16 days for UK system and 42 days for France system
ATSA $DT_{50\ whole\ system}$: 22 days for UK system and 71 days for France system

Minimal mineralisation was observed with a maximum of 2% AR in UK system and 0.8% AR in France system after 101 days.

Figure 2: Proposed degradation pathway of pyroxsulam in water-sediment systems under aerobic conditions (taken from DAR, Volume 3, Annex B8: Environmental Fate and Behaviour – January 2012)



Pyroxsulam (XDE-742)

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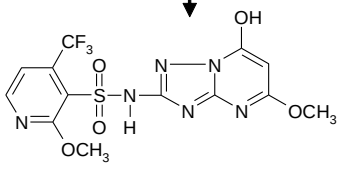
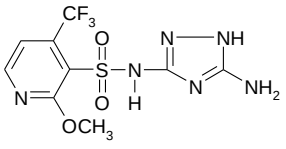


7-OH-XDE-742



XDE-742 ATSA

CO_2 (minor) + Bound residues

5.1.3 Summary and discussion of degradation

Pyroxsulam is considered hydrolytically stable.

Pyroxsulam is susceptible to photodegradation. The experimental DT_{50} in sterile pure water was 4.1 days at 40^{o}N in summer sunlight. Two degradants were identified with DT_{50} values of 32 and 21-41 days 40^{o}N in summer sunlight. The actual degree of photodegradation in the aquatic environment depends on local conditions and seasons. Therefore, in reality the potential for aquatic photolysis is likely to be limited.

In a ready biodegradation study a maximum of 23% degradation was observed over 28 days and pyroxsulam is considered to be 'not readily degradable'.

In an aerobic water-sediment study pyroxsulam was observed to dissipate from the water column to sediment in two systems where subsequent decline was also noted. Estimated whole system dissipation DT_{50} values for pyroxsulam were between 12 and 24 days. Two key degradants were observed with whole system DT_{50} values of 16 to 42 and 22 to 71 days. Minimal mineralisation (maximum 2% AR by day 101) was observed.

Overall, the degradation information does not provide sufficient data to show pyroxsulam is ultimately degraded within 28 days (equivalent to a half-life <16 days) or transformed to non classifiable products. Consequently, pyroxsulam is considered not rapidly degradable for the purpose of classification and labelling.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Following OECD Test Guideline 106 and GLP, a soil adsorption study (Smith, 2004) is available investigating the adsorption of pyroxsulam. The study used 10 soils from the UK and Germany and ^{14}C-pyroxsulam. Soil pH ranged from 5.4 to 7.9 and organic carbon from 0.8 to 3.8%. When normalised for organic carbon, adsorption was observed to be pH dependant with increasing adsorption with decreasing soil pH. The K_{oc} values ranged between 3.62 and 83.86 ml/g. This equates to log K_{oc} values between 0.56 and 1.92.

5.2.2 Volatilisation

Experimental data (Madsen and Kastel, 2003) indicate the vapour pressure for pyroxsulam is $<1 \times 10^{-7}$ Pa at 20 °C following OECD Test Guideline 104. The Henry's Law Constant (Madsen, 2006b) was calculated to be $<1.36 \times 10^{-8}$ Pa m^3 mol^{-1} at 20 °C, pH 7 indicating pyroxsulam is unlikely to partition from the water phase to air.

5.2.3 Distribution modelling

Not relevant for classification and labelling.

5.3 Aquatic Bioaccumulation

Table 23: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient *n*-octanol/water (shake flask method)	Log K_{ow} 1.08 at pH 4, 20°C Log K_{ow} -1.01 at pH 7, 20°C Log K_{ow} -1.6 at pH 9, 20°C	Valid	Turner, (2004b)

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

No data available.

5.3.1.2 Measured bioaccumulation data

No data available.

5.3.2 Summary and discussion of aquatic bioaccumulation

The experimental log K_{ow} for pyroxsulam is 1.08 at pH 4 and -1.01 at pH 7 and 20°C (Turner, 2004b). The lower pH 4 value is anticipated to represent the non-ionised form while the pH 7 value is anticipated to reflect a predominantly ionised form likely to be present at environmentally relevant pH. Overall, the log K_{ow} is below the CLP logK_{ow} trigger value of ≥ 4 intended to identify substances with a potential to bioaccumulate. Given this low value, an experimental BCF study was not required.

5.4 Aquatic toxicity

A summary of available valid information on the aquatic toxicity of pyroxsulam (98% purity) is presented in Table 24. Where available, a summary of valid information for degradants is also included in Annex II, Table 1.

Studies were reviewed under Directive 91/414/EEC and considered valid and reliable. Further details are presented for studies conducted on the active substance pyroxsulam but not for its degradants as these are all of similar or lower toxicity and are not considered further for the environmental hazard classification of pyroxsulam.

The water solubility of pyroxsulam is pH dependant (16.4 mg/l at pH4, 3.2 g/l at pH 7 and 13.7 g/l at pH 9. The water pH during aquatic testing is noted in Table 24. Given experimental pH values, this is not anticipated to have affected key study results.

Table 24: Summary of relevant information on aquatic toxicity for pyroxsulam (XDE-742)

Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg a.s./l)	
Acute toxicity to fish OECD Guideline 203, GLP, purity: 98%	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Static pH 7.5 to 8.5	96 hours	LC_{50}	>87 (mm)	Zok, 2003c
Acute toxicity to fish OECD Guideline 203, GLP, purity: 98%	Fathead Minnow (*Pimephales promelas*)	Mortality	Static pH 7.5 to 8.5	96 hours	LC_{50}	>94.4 (mm)	Zok, 2003d
Fish Early Life-Stage (FELS) toxicity OECD Guideline 210, GLP, purity: 98%	Fathead Minnow (*Pimephales promelas*)	Time to hatch, hatching success, survival and growth (length, wet weight and dry weight)	Flow-through pH 7.0 to 7.5	40 days	NOEC	10.1 (mm)	Marino *et al*, 2005
Daphnia sp Acute Immobilisation OECD Guideline, 202 GLP, purity: 98%	*Daphnia magna*	Acute immobilisation	Static pH 7.2 to 8.0	48 hours	EC_{50}	>100 (mm)	Marino *et al*, 2004
Daphnia magna Reproduction OECD Guideline 211, GLP, purity: 98%	*Daphnia magna*	Survival; reproduction; growth	Flow-through pH 7.2 to 7.9	21 days	NOEC	10.4 (mm)	Marino *et al*, 2005
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity: 98%	*Pseudo-kirchneriella subcapitata**	Cell multiplication inhibition	Static pH 7.5-7.7 to 8.6-10.5	72 hours	ErC_{50} NOErC	0.924 (mm) 0.055 (mm)	Hancock *et al*, 2004
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity: 98%	*Anabaena flos-aquae*	Cell multiplication inhibition	Static pH 5.0-7.4 to 5.1-7.6	72 hours	ErC_{50} NOErC	41 (mm) 13 (mm)	Hoberg, 2005a
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity: 98%	*Skeletonema costatum*	Cell multiplication inhibition	Static pH 7.9-8.2 to 8.2-8.7	96 hours	ErC_{50} NOErC	59 (mm) 3.4 (mm)	Hancock *et al*, 2005
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity: 98%	*Navicula pelliculosa*	Cell multiplication inhibition	Static pH 6.8-7.1 to 6.8-9.2	72 hours	ErC_{50} NOErC	6.9 (mm) 4 (mm)	Hoberg, 2005b
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity: 98%	*Lemna gibba*	Growth	Semi-static pH 7.5-7.9 to 7.1-8.3	7 days	ErC_{50} NOErC	**0.00388 (mm)** **0.000681 (mm)**	Hancock *et al*, 2005b

Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg a.s./l)	
Sediment-water toxicity Test. OECD Guideline 219, purity: 98%	*Chrionomus riparius*	Emergence and survival	Static, spike water pH 7.4-8.3	28 days	NOEC	100 (n)	Henry *et al*, 2005

Notes:
mm refers to results based on mean measured test concentrations
n refers to nominal concentrations
*formerly *Selenastrum capricornutum*

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Two acute toxicity to fish studies using pyroxsulam (purity >98%) are available following GLP and OECD Guideline 203.

Study 1 (Zok, 2003c)

Using Rainbow Trout (*Oncorhynchus mykiss*), a static limit test was performed using the nominal concentration 100 mg/l. Study conditions were within the test guideline range and validation criteria were met. Analytical verification was >86% of nominal. The study 96-h LC_{50} was >100 mg a.s./l (nominal), >87 mg a.s./l (mean measured). The study 96-h NOEC was 100 mg a.s./l (nominal), 87 mg a.s./l (mean measured).

Study 2 (Zok, 2003d)

Using Fathead Minnow (*Pimephales promelas*) a static limit test was performed using the nominal concentration 100 mg/l. Aside from one dissolved oxygen measurement dropping to 58% below the 60% guideline, study conditions were within the test guideline range and validation criteria were met. The study 96-h LC_{50} was >100 mg a.s./l (nominal), >94.4 mg a.s./l (mean measured). The study 96-h NOEC was 100 mg a.s./l (nominal), 94.4 mg a.s./l (mean measured).

5.4.1.2 Long-term toxicity to fish

A 40-day flow-through chronic toxicity to fish study (Marino *et al*, 2005) using pyroxsulam following GLP and OECD Guideline 210 is available. The study used Fathead Minnow (*Pimephales promelas*) and the following endpoints: time to hatch, hatching success, survival and growth. The nominal exposure range was 0.778, 1.3, 2.16, 3.69, 6 and 10 mg a.s./l. Results were based on mean measured values: 0.836, 1.28, 2.23, 3.62, 6.11 and 10.1 mg a.s./l. Validity criteria were met and the test is considered reliable. Significant effects were not observed for any parameter. The study 40-d NOEC was 10.1 mg a.s./l reflecting the highest mean measured exposure concentration.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

A static acute toxicity to *Daphnia magna* study (Marino *et al*, 2004) using pyroxsulam is available following GLP and OECD Guideline 202. The nominal exposure range was 13, 21.6, 36, 60 and 100 mg a.s./l. Results were based on mean measured values: 12.2, 20.6, 34.9, 58.8 and 100 mg a.s./l. Validity criteria were met and the test is considered reliable. As no significant effects were observed, the study 48-h LC_{50} was >100 mg a.s./l based on mean measured. The study 48-h NOEC was 100 mg a.s./l based on mean measured.

5.4.2.2 Long-term toxicity to aquatic invertebrates

A semi-static chronic toxicity to *Daphnia magna* study (Marino *et al*, 2005) using pyroxsulam is available following GLP and OECD Guideline 211. The study assessed the following endpoints: survival, reproduction, length and weight. The nominal exposure range was 0.0313, 0.625, 1.25, 2.5, 5 and 10 mg a.s./l. Results were based on mean measured values: 0.0353, 0.701, 1.37, 2.66, 5.27 and 10.4 mg a.s./l. Validity criteria were met and the test is considered reliable. Significant effects were not observed for any parameter. The study 21-d NOEC was 10.4 mg a.s./l reflecting the highest mean measured exposure concentration.

5.4.3 Algae and aquatic plants

Algae:

Four algal growth inhibition studies using pyroxsulam are available.

Study 1 (Hancock *et al*, 2004)

A static algal growth inhibition test using pyroxsulam (purity 98%) and *Pseudokirchneriella subcapitata* is available following GLP and OECD Guideline 201. The nominal exposure range was 0.0313, 0.0625, 0.125, 0.25, 0.5, 1 and 2 mg a.s./l. Results were based on mean measured values: 0.0261, 0.0550, 0.126, 0.252, 0.503, 1.01 and 2.04 mg a.s./l. Validity criteria were met and the test is considered reliable. The 72-h E_rC_{50} was 0.924 mg a.s./l and the 72-hour NOE_rC was 0.055 mg a.s./l based on mean measured concentrations.

Study 2 (Hoberg *et al*, 2005a)

A static algal growth inhibition test using pyroxsulam (purity 98%) and the cyanobacteria *Anabaena flos-aquae* is available following GLP and OECD Guideline 201. The nominal exposure range was 0.041, 1.0, 2.6, 6.4, 16, 40 and 100 mg a.s./l. Results were based on mean measured values: 0.036, 0.89, 2.2, 5.4, 13, 28 and 85 mg a.s./l. Validity criteria were met and the test is considered reliable. Initial pH values were 5 to 7.4 and final pH values 5.1 to 7.6. Study observations did not include undissolved material and the lower range pH is not anticipated to have affected study results. The 72-h E_rC_{50} was 41 mg a.s./l and the 72-hour NOE_rC was 13 mg a.s./l based on mean measured concentrations.

Study 3 (Hancock *et al*, 2005)

A static algal growth inhibition test using pyroxsulam (purity 98%) and diatom *Skeletonema costatum* is available following GLP and OECD Guideline 201. The nominal exposure range was 3.13, 6.25, 12.5, 25, 50 and 100 mg a.s./l. Results were based on mean measured values: 3.4, 6.8, 13.6, 26.7, 52.8 and 105 mg a.s./l. Validity criteria were met and the test is considered reliable. The

96-h E_rC_{50} was 59 mg a.s./l and the 96-hour NOE_rC was 3.4 mg a.s./l based on mean measured concentrations.

Study 4 (Hoberg, 2005b)

A static algal growth inhibition test using pyroxsulam (purity 98%) and *Navicula pelliculosa* is available following GLP and OECD Guideline 201. The nominal exposure range was 0.1, 0.26, 0.64, 1.6, 4 and 10 mg a.s./l. Results were based on mean measured values: 0.1, 0.29, 0.67, 1.7, 4 and 10 mg a.s./l. Validity criteria were met and the test is considered reliable. The 72-h E_rC_{50} was 6.9 mg a.s./l and the 72-hour NOE_rC was 4 mg a.s./l based on mean measured concentrations.

Aquatic plants:

A semi-static 7-day toxicity to *Lemna gibba* study (Hancock *et al*, 2005b) using pyroxsulam is available following GLP and OECD Guideline 221.

Exposure solutions were prepared with the aid of the solvent DMF (0.1ml/l) and a solvent control was included. The nominal exposure range was 0.313, 0.625, 1.25, 2.5, 5 and 10 µg a.s./l. Analytical measurement used liquid chromatography positive electrospray ionization mass spectrometry (LC/PESI-MS). Results were based on mean measured fresh media concentrations from days 0, 3 and 5 as pyroxsulam was considered stable based on analytical concentrations of expired solutions which were 89.5 to 117% nominal. This resulted in a mean measured test concentration range of: 0.335, 0.681, 1.34, 2.81, 5.23 and 10.3 µg/l.

The study pH was 7.5 – 7.9 initially and 7.1 – 8.3 for expired solutions with plants. Validity criteria were met and the test is considered reliable. The study endpoints were percentage reduction in frond number, biomass, growth rate based on frond number and growth rate based on biomass. Table 25 shows growth rates in relation to exposure concentrations. Table 26 shows effect concentrations and NOEC values for assessed endpoints.

Table 25: Summary of pyroxsulam (XDE-742) effects on growth rate of the aquatic plant, *Lemna gibba*

Mean Measured Concentration (µg a.s./L)	Mean growth rate per day	% Difference [a]
Control	0.404	---
Vehicle Control	0.393	---
Pooled Control	0.398	---
0.335	0.411	-3
0.681	0.405	-2
1.34	0.376*	5
2.81	0.249*	37
5.23	0.131*	67
10.3	0.0844*	79

[a] Percent difference as compared to the pooled control was determined on day 7

* Growth was significantly less than the pooled control (Dunnett's test, p = 0.05).

Table 26: Summary of pyroxsulam (XDE-742) effect concentrations for the aquatic plant, *Lemna gibba* - based on mean measured test concentrations over 7 days exposure

Endpoint	Parameter Effect Concentration as µg a.s./L		
	EC_{50}	95% confidence limit	NOEC
Frond Number	2.57	1.16 - 5.70	0.681
Growth Rate	3.88	1.68 - 8.97	0.681
Biomass as Dry Weight	3.82	2.23 - 6.56	0.681

The key growth rate endpoint for acute hazard classification purposes is the 7-d E_rC_{50} of 0.00388 mg a.s./l (95% confidence intervals 0.00168 to 0.00897 mg a.s./l) based on mean measured. The key growth rate endpoint for chronic classification is the 7-d NOE_rC of 0.000681 mg a.s./l, also based on mean measured test concentrations.

5.4.4 Other aquatic organisms (including sediment)

A static 28-day toxicity to *Chrironomus riparius* (midge larvae) study (Henry *et al*, 2005) is available using pyroxsulam (98% purity) following OECD Guideline 219. The nominal exposure range was 6.25, 12.5, 25, 50 and 100 mg a.s./l. Exposure was via the water phase and a sediment phase was present. Concentrations of pyroxsulam (as a percentage of nominal) in the water phase were 104% on day 0, 86.3% on day 7 and 99% on day 28. Validity criteria were met and the test is considered reliable. No statistical differences were observed between exposure and control systems. The 28-day NOEC was 100 mg a.s./l based on nominal.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

For the purpose of classification, pyroxsulam is considered not rapidly degradable.

The experimental log K_{ow} for pyroxsulam is 1.08 at pH 4 and -1.01 at pH 7 and 20^{o}C. The lower pH 4 value is anticipated to represent the non-ionised form while the pH 7 value is anticipated to reflect a predominantly ionised form likely to be present at environmentally relevant pH. Overall, the log K_{ow} is considered to be below the CLP log K_{ow} trigger value of ≥ 4 intended to identify substances with a potential to bioaccumulate.

Identified degradants are of similar or lower toxicity to the parent substance (see Annex II) and are not considered further for the environmental classification of pyroxsulam.

Aquatic acute toxicity data on pyroxsulam are available for fish, invertebrates, algae and aquatic plants. Aquatic plants are the most acutely sensitive trophic group. The lowest $L(E)C_{50}$ value is a 7-day E_rC_{50} of 0.00388 mg/l for *Lemna minor* in the range 0.001 to $\leq$0.01 mg/l. On this basis pyroxsulam should be classified as Aquatic Acute 1 with an M factor of 100.

Adequate chronic toxicity data on pyroxsulam are available for fish, invertebrates, algae and aquatic plants. The lowest value is a 7-day NOE_rC for *Lemna minor* of 0.0007 mg/l. Given this is in the range 0.0001 to $\leq$0.001 mg/l and the substance is considered non-rapidly degradable, pyroxsulam should be classified as Aquatic Chronic 1 with an M factor of 100.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Aquatic Acute 1; H400: Very toxic to aquatic life
Acute M factor = 100
Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects
Chronic M factor = 100

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Environmental fate

Standard tests were used in GLP laboratories: all studies were valid.

1. Aqueous hydrolysis study in accordance with (i) US EPA Guideline Subdivision N 161-1 and (ii) SETAC Guideline Part 1, Section 9: no significant degradation was observed and analysis showed 100% radioactivity as pyroxsulam at study termination.

The DS concluded that pyroxsulam is hydrolytically stable.

2. Two aqueous photolysis study in accordance with (i) US EPA Guideline Subdivision N 161-2 and (ii) SETAC Guideline Part 1, Section 10.1, resulted a DT_{50} = 4.1 days at 40 °N in summer sunlight. In the same conditions, the DT_{50} of the degradation products was between 21 and 41 days. Therefore the potential for aquatic photolysis was judged as limited by the DS.

3. Biodegradation screening test (OECD TG 301B) measured a maximum of 19% and 23% degradation over 28 days in duplicate samples, thus pyroxsulam was considered as not readily biodegradable.

4. Two simulation studies, according to guidelines (i) SETAC Guideline Part 1, Section 8.2 and (ii) BBA Guideline Part IV, Section 5 demonstrated:

- Decrease in water: from 84–103.4% AR to 14.4 and 22.1% AR by day 101;
- Increase in sediment: from 0.8–16.1% AR to 17.2 and 42% AR by day 75;

Measured data showed that dissipation but no mineralisation occurred:

- Dissipation DT_{50} based on whole system: 12 to 24 days;
- Degradation DT_{50} based on whole system: 17 to 33 days;
- Mineralisation 0.8–2% AR after 101 days.

5. Bioaccumulation: the Log K_{ow} was measured at 20°C in a study performed according to ECC Method A.5. The values did not indicate bioaccumulative potential:

log K_{ow}: 1.08 at pH 4, 20°C
log K_{ow}: –1.01 at pH 7, 20°C
log K_{ow}: –1.6 at pH 9, 20°C.

Overall, pyroxsulam was characterised by DS as not readily degradable and having no bioaccumulative potential.

Aquatic toxicity

98% purity substance was tested in GLP laboratories. The mean measured test concentrations are given below.

Fish lethality, daphnid immobilisation, freshwater algal growth inhibition with 4 algal species and duckweed growth inhibition were measured.

1. 96 h acute fish toxicity measured by OECD TG 203 using two species, gave the following results:
   - *Oncorhynchus mykiss*: LC_{50} >87 mg/L and
   - *Pimephales promelas*: LC_{50} >94 mg/L;

2. 48 h acute daphnid immobilisation, measured by OECD TG 202:
   - *Daphnia magna* LC_{50} >100 mg/L;

3. Freshwater algal growth inhibition, measured by OECD TG 201 on 4 algal species:
   - *Anabaena flosaquae*: 72 h E_rC_{50} = 41 mg/L;
   - *Navicula pelliculosa*: 72 h E_rC_{50} = 6.9 mg/L;
   - *Pseudokirchneriella subcapitata**:72 h E_rC_{50} = 0.924 mg/L;
   - *Skeletonem acostatum*: 96 h E_rC_{50} = 59 mg/L.

4. Freshwater algal growth inhibition, measured by OECD TG 201 on 4 algal species:
   - *Anabaena flosaquae*: 72 h NOE_rC = 13 mg/L;
   - *Navicula pelliculosa*: 72 h NOE_rC = 4 mg/L;
   - *Pseudokirchneriella subcapitata*: 72 h NOE_rC = 0.055 mg/L;
   - *Skeletonema costatum*: 96 h NOE_rC = 3.4 mg/L.

5. Duckweed growth inhibition test OECD TG 221
   - *Lemna gibba*: 7 days E_rC_{50} = **0.00388 mg/L,**
   - *Lemna* gibba: 7 days chronic NOE_rC = **0.000681 mg/L**.

The lowest E_rC_{50} value, measured with *Lemna gibba*, is the basis for the aquatic acute classification proposed by DS. Based on the lowest acute toxicity results, the DS proposed to classify pyroxsulam as Aquatic Acute Cat. 1, with an M factor of 100.
Based on lowest chronic toxicity results, the DS proposed to classify pyroxsulam as Aquatic Chronic Cat. 1, with an M factor of 100.

Comments received during public consultation

One supportive comment arrived from one member state, agreeing with the Aquatic Acute and Chronic classifications and M-factors.

Assessment and comparison with the classification criteria

Environmental fate

- Pyroxsulam is considered hydrolytically stable.
- Pyroxsulam is susceptible to photodegradation, but in reality the potential for aquatic photolysis is likely to be limited.
- In a ready biodegradation study a maximum of 23% degradation was observed over 28 days therefore pyroxsulam is considered to be not readily degradable.
- In aerobic water–sediment simulation studies pyroxsulam dissipated from the water column to sediment and only a minimal mineralisation (maximum 2% AR by day 101) was observed. The data do not provide evidence of ultimate degradation within 28 days.
- Log K_{ow} does not indicate aquatic bioaccumulation potential, given that K_{ow} <4, under environmentally relevant conditions.

In summary, pyroxsulam is considered not rapidly degradable for the purpose of classification and labelling.

Aquatic toxicity

Aquatic acute toxicity data on pyroxsulam are available for fish, invertebrates, algae and the aquatic plant of *Lemna gibba*.

The lowest acute toxicity value is represented by the 7-day **E_rC_{50} of 0.00388 mg/L** for *Lemna gibba.*

The **E_rC_{50}** is in the range 0.001 < 0.00388 < 0.01 mg/L, which corresponds to a multiplying factor of 100 in Table 4.1.3 of the CLP Regulation.

On this basis pyroxsulam is classified as **Aquatic Acute 1** with an **M factor of 100**(Hazard statement code: H400).

Aquatic chronic toxicity data on pyroxsulam are available for fish, invertebrates, algae and aquatic plants.

The lowest chronic toxicity value is a 7-day **NOE_rC of 0.0007 mg/L** for *Lemna gibba.*

The **NOE_rC** is in the range 0.0001 < 0.0007 < 0.001 mg/L, the substance is non readily biodegradable, hence a multiplying factor of 100 is appropriate.

Identified degradants are of similar or lower toxicity to the parent substance thus these are not considered for the environmental classification of pyroxsulam.

Given that pyroxsulam proved to be non-rapidly degradable, it should be classified as **Aquatic Chronic 1** with an **M factor of 100** (Hazard statement code: H410).

Overall, RAC agrees to classify pyroxsulam as **Aquatic Acute 1** with an **M factor of 100**, and **Aquatic Chronic 1** with an **M factor of 100**.

6 OTHER INFORMATION

None

7 REFERENCES

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Toxicology and metabolism

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KIIA 5.4.5	Beevers, C.	2006	XDE-742: Measurement of unscheduled DNA synthesis in mouse liver using an in vivo/in vitro procedure DAS Report No.: 060105 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N

DAR Reference	**Author(s)**	**Year**	**Title** **Source (where different from the Company),** **Company, Report Number,** **GLP or GEP status (where relevant),** **Published or not**
KIIA 5.5.1	Stebbins, K.E.; Brooks, K.J.	2005	XDE-742: 2-Year Chronic Toxicity/Oncogenicity and Chronic Neurotoxicity Study in Fischer 344 DAS Report No.: 31014 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 5.5.3	Johnson, K.A.; Dryzga, M.D.; Yano, B.L.	2005	XDE-742: 18-Month Dietary Oncogenicity Study in CD-1 Mice DAS Report No.: 31015 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 5.6.1/02	Carney, E.W.; Zablotny, C.L.; Stebbins, K.E.	2005	XDE-742: 2-Generation Dietary Reproductive Toxicity Study in CD Rats DAS Report No.: 41012 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
N/A	Giknis, M; Clifford, C	2010	Spontaneous Neoplastic Lesions in the Crl:CD1 (ICR) Mouse in Control Groups from 18 Month to 2 Year Studies Published (Y/N): Y

Environmental Hazards

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not
KIIA 7.7	Schwarz, M.	2003	XDE-742/BAS 770 H Determination of the Biodegradability in the CO2-Evolution Test BASF, Germany DAS Report No.: 03/0298/22/1 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 7.8.3	Yoder, R.N. Cook, W.L. Meitl, T.J. Balcer, J.L. Linder, S.J.	2006c	Aerobic Aquatic Degradation of XDE-742 in Two European Sediment and Pond Water Systems Dow AgroSciences LLC DAS Report No.: 30076 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
	Smith, J.K.	2004	Soil Batch Equilibrium Adsoroption/Desorption of 14C-XDE-742 Dow AgroSciences LLC DAS Report No.: 30069 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.2.1.1	Zok, S.	2003c	XDE-742/BAS 770 H Acute Toxicity Study on the Rainbow Trout (Onchrhynchus Mykiss) in a Static System over 96 Hours BASF, Germany DAS Report No.: 35031 (Masterfile Number): 144912 GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.2.1.2	Zok, S.	2003d	XDE-742/BAS 770 H Acute Toxicity Study on the Fathead Minnow (Pimephales Promelas) in a Static System over 96 Hours BASF, Germany DAS Report No.: 35032 (Masterfile Number): 144913 GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.2.1.3/01	Sayers, L.E.	2006a	7-OH Metabolite of XDE-742 - Acute Toxicity to Rainbow Trout (Oncorhynchus Mykiss) Under Static Conditions Springborn Smithers Laboratories DAS Report No.: 50165 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.2.1.3/02	Marino, T.A.; Arnold, B.H.; Sushynski. J.M.; Yaroch, A.M.	2006	ATSA Metabolite of XDE-742: An Acute Toxicity Study with the Rainbow Trout, Oncorhynchus Mykiss The Dow Chemical Company DAS Report No.: 61010 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N

Annex Point/ Reference Number	**Author(s)**	**Year**	**Title** **Source (where different from the Company),** **Company, Report Number,** **GLP or GEP status (where relevant),** **Published or not**
KIIA 8.2.4	Marino, T.A.; Hales, C.A.; McClymont, E.L.; Yaroch, A.M.	2005	XDE-742: Toxicity to the Early Life Stages of the Fathead Minnow, Pimephales, promelas The Dow Chemical Company DAS Report No.: 51007 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.3.1.1/01	Marino, T.A.; McClymont, E.L.; Najar, J.R.	2004	XR-742: An Acute Toxicity Study with the Daphnid, Daphnia Magna The Dow Chemical Company DAS Report No.: 41022 (Masterfile Number): 148998 GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.3.1.1/02	Sayers, L.E.	2006b	7-OH Metabolite of XDE-742 - Acute Toxicity to Water Fleas, Daphnia Magna, Under Static Conditions Springborn Smithers Laboratory DAS Report No.: 50164 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.3.1.1/03	Marino, T.A.; Arnold, B.H.; Najar, J.R.; Sushynski, J.M.	2006	ATSA Metabolite of XDE-742: An Acute Toxicity Study with the Daphnid, Daphnia Magna The Dow Chemical Company DAS Report No.: 61005 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.3.2.1	Marino, T.A.; McClymonty, Najar, J.R.	2005	XDE-742: A 21-Day Chronic Toxicity Study with the Daphnid, Daphnia magna The Dow Chemical Company DAS Report No.: 41023 (Masterfile Number): 205756 GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.4.1/01	Hancock, G.A.; McClymont, E.L.; Staley, J.L.	2004	XDE-742: Growth Inhibition Test with the Freshwater Green Alga, Pseudokirchneriella subcapitata The Dow Chemical Company DAS Report No.: 41054 (Masterfile Number): 149174 GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.4.1/02	Hoberg, J.R.	2005a	XDE-742 - Growth Inhibition Test with the Freshwater Bluegreen Alga (Anabaena flos aquae) Springborn Smithers Laboratories, 790 Main Street, Wareham, DAS Report No.: 50284 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N

Annex Point/ Reference Number	**Author(s)**	**Year**	**Title** **Source (where different from the Company),** **Company, Report Number,** **GLP or GEP status (where relevant),** **Published or not**
KIIA 8.4.1/03	Hancock, G.A.; Hales, C.A.; McClymont, E.L.; Najar, J.R.	2005	XDE-742: Growth Inhibition of the Saltwater Diatom, Skeletonema costatum The Dow Chemical Company DAS Report No.: 51039 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.4.1/04	Hoberg, J.R.	2005b	XDE-742 - Growth Inhibition Test with the Freshwater Diatom (Navicula pelliculosa) Springborn Smithers Laboratories, 790 Main Street, Wareham, DAS Report No.: 50283 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.4.1/05	Hoberg, J.R.	2005c	XDE-742 Sulfinic Acid Metabolite Acute Toxicity to the Freshwater Green Alga, Pseudokirc hneriella subcapitata Springborn Smithers Laboratories, USA DAS Report No.: 50110 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.4.1/06	Hoberg, J.R.	2005d	7-OH Metabolite of XDE-742 - Acute Toxicity to the Freshwater Green Alga, Pseudokirchneriella Subcapitata Springborn Smithers Laboratories DAS Report No.: 50108 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.4.1/07	Hancock, G.A.; Arnold, B.H.; Najar, B.S.; Sushynski, J.M.	2006a	ATSA Metabolite of XDE-742 Growth Inhibition Test with the Freshwater Green Alga, Pseudokirchneriella Subcapitata The Dow Chemical Company DAS Report No.: 61002 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.4.1/08	Hoberg, J.R.	2006a	5-OH Metabolite of XDE-742 - Acute Toxicity to the Freshwater Green Alga, Pseudokirchneriella Subcapitata Springborn Smithers Laboratory DAS Report No.: 50107 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.4.1/09	Hoberg, J.R.	2006b	5,7-Di-OH Metabolite of XDE-742 - Acute Toxicity to the Freshwater Green Alga, Pseudokirchneriella Subcapitata Sprinborn Smithers Laboratories DAS Report No.: 50109 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N

Annex Point/ Reference Number	**Author(s)**	**Year**	**Title** **Source (where different from the Company),** **Company, Report Number,** **GLP or GEP status (where relevant),** **Published or not**
KIIA 8.4.1/10	Hoberg, J.R.	2006c	6-C1-7-OH Metabolite of XDE-742 - Acute Toxicity to the Freshwater Green Alga, Pseudokirchneriella Subcapitata Springborn Smithers Laboratory DAS Report No.: 50112 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.4.1/11	Hoberg, J.R.	2006d	ADTP Metabolite of XDE-742 - Acute Toxicity to the Freshwater Green Alga, Pseudokirchneriella Subcapitata Springborn Smithers Laboratories DAS Report No.: 50111 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.4.1/12	Aufderheide, J.	2007	Sulfonamide Metabolite of XDE-742: Growth Inhibition Test with the Unicellular Green Alga, *Pseudokirchneriella subcapitata* ABC Laboratories DAS Report No.: 070314 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.5.2/01	Henry, K.S.; McClymont, E.L.; Najar, J.R.	2005	XDE-742: 28-Day Chronic Toxicity Study with the Midge, Chironomus riparius, Using Spiked Water in a Sediment-Water Exposure System The Dow Chemical Company DAS Report No.: 41061 (Masterfile Number): 149503 GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.5.2/02	Putt, A.E.	2006	7-OH Metabolite of XDE-742 - Chironomid Toxicity Test with Midge (Chironomus Riparius) Under Static Conditions Using Spiked Water Springborn Smithers Laboratories DAS Report No.: 50166 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.6/01	Hancock, G.A.; McClymont, E.L.; Najar, J.R.	2005	XDE-742: Growth Inhibition Test with the Aquatic Plant Duckweed, Lemna gibba The Dow Chemical Company DAS Report No.: 41124 (Masterfile Number): 207218 GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.6/03	Hoberg, J.R.	2005e	XDE-742 Sulfinic Acid Metabolite Toxicity to Duckweed, Lemna Gibba Springborn Smithers Laboratories, USA DAS Report No.: 50122 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N

Annex Point/ Reference Number	**Author(s)**	**Year**	**Title** **Source (where different from the Company),** **Company, Report Number,** **GLP or GEP status (where relevant),** **Published or not**
KIIA 8.6/04	Hoberg, J.R.	2006e	7-OH Metabolite of XDE-742 - Toxicity to Duckweed, Lemna Gibba Springborn Smithers Laboratories DAS Report No.: 50119 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.6/05	Hancock, G.A.; Arnold, B.H.; Najar, J.R.; Sushynski, J.M.	2006b	ATSA Metabolite of XDE-742: Growth Inhibition Test with the Aquatic Plant Duckweed, Lemna Gibba The Dow Chemical Company DAS Report No.: 61006 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.6/06	Hoberg, J.R.	2006f	5-OH Metabolite of XDE-742 - Toxicity to Duckweed, Lemna Gibba Springborn Smithers Laboratories DAS Report No.: 50120 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.6/07	Hoberg, J.R.	2006g	5,7-Di-OH Metabolite of XDE-742 - Toxicity to Duckweed, Lemna Gibba Springborn Smithers Laboratories DAS Report No.: 50121 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.6/08	Hoberg, J.R.	2006h	6-C1-7-OH Metabolite of XDE-742 - Toxicity to Duckweed, Lemna Gibba Springborn Smithers Laboratories DAS Report No.: 50124 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.6/09	Hoberg, J.R.	2006i	ADTP Metabolite of XDE-742 - Toxicity to Duckweed, Lemna Gibba Springborn Smithers Laboratories DAS Report No.: 50123 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N
KIIA 8.6/10	Hicks, S.L.	2007	Sulfonamide Metabolite of XDE-742: Growth Inhibition Test with the Freshwater Aquatic Plant, Duckweed, *Lemna gibba* ABC Laboratories DAS Report No.: 070315 (Masterfile Number): n/a GLP/GEP (Y/N): Y Published (Y/N): N

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Caldwell (1999) Review of Mononuclear Cell Leukemia in F-344 Rat Bioassays and Its Significance to Human Cancer Risk: A Case Study Using Alkyl Phthalates. Regul. Toxicol. Pharmacol. 30(1): 45–53

Gopinath, C. (15th September, 2008). Fischer rat leukaemia reported in an XDE-742 (pyroxsulam) carcinogenicity study.

Haseman et al., (2003) Effect of Diet and Animal Care/Housing Protocols on BodyWeight, Survival, Tumor Incidences, and Nephropathy Severity of F344 Rats in Chronic Studies. Toxicol. Pathol. 31(6):674–81

Muller (2005) Mononuclear Cell Leukaemia in the F344 rat strain. Factsheet FSV-016/00 RIVM in Factsheets for the (eco)toxicological risk assessment strategy of the National Institute for Public Health and the Environment Part V. Report 601516013/2005, 43-55.

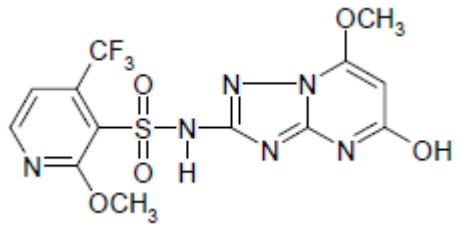
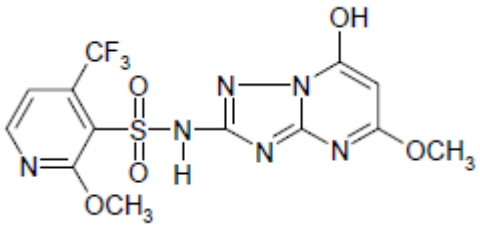
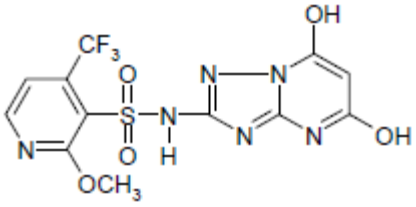
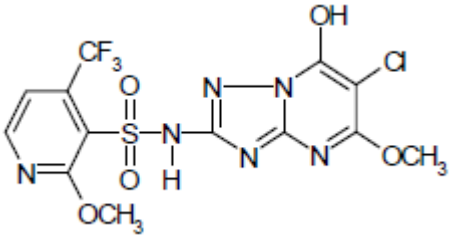
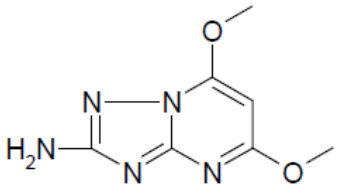
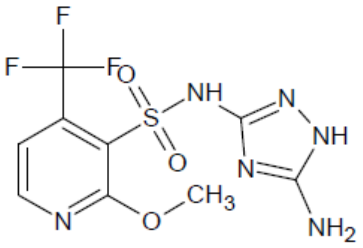
8 ANNEXES

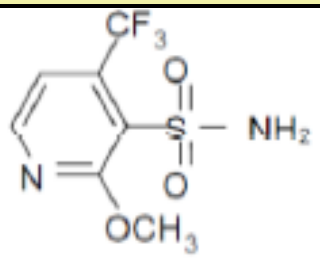
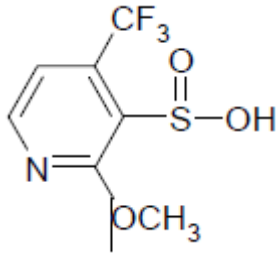
Annex I - Degradant code, chemical name and structure.

Annex II - Aquatic toxicity data for pyroxsulam degradants

ANNEX I – Degradant code, chemical name and structure.

Table 1: Identity of degradants

Name / code name	Chemical name	Structural formula
5-OH-pyroxsulam 5-OH-XDE-742	*N*-(5-hydroxy-7-methoxy[1,2,4] triazolo[1,5-*a*]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide	
7-OH-pyroxsulam 7-OH-XDE-742	*N*-(7-hydroxy-5-methoxy[1,2,4] triazolo[1,5-*a*]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide	
5,7-OH-pyroxsulam 5,7-diOH-XDE-742	*N*-(5,7-dihydroxy[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide	
6-Cl-7-OH-pyroxsulam 6-Cl-7-OH-XDE-742	*N*-(6-chloro-7-hydroxy-5-methoxy [1,2,4] triazolo[1,5-*a*]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl) pyridine -3-sulfonamide	
ATDP	5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-amine	
ATSA	*N*-(5-amino-1*H*-1,2,4-triazol-3-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide	

Name / code name	Chemical name	Structural formula
Pyridine sulfonamide	2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide (IUPAC)	
Pyridine sulfinic acid	2-methoxy-4-(trifluoromethyl)pyridine-3-sulfinic acid (IUPAC) 3-pyridinesulfinic acid, 2-methoxy-3-trifluoromethyl (CAS)	

ANNEX II – Aquatic toxicity data for pyroxsulam degradants

Table 1: Summary of relevant information on aquatic toxicity for pyroxsulam degradants

Degradant / Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/l)	
7-OH-XDE-742							
Acute toxicity to fish OECD Guideline 203, GLP, purity 99%)	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Static	96 hours	LC_{50}	>120 (mm)	Sayers, 2006a
Daphnia sp Acute Immobilisation OECD Guideline, 202, GLP, purity 99%)	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	>99 (mm)	Sayers, 2006b
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity 96%)	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	72 hours	ErC_{50} NOErC	65 (mm) 16 (mm)	Hoberg, 2005d
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity 99%)	*Lemna gibba*	Growth	Semi-static	7 days	ErC_{50} NOErC	4.0 (mm) 0.74 (mm)	Hoberg, 2006e
Sediment-water toxicity Test. OECD Guideline 219, purity: 99%	*Chrionomus riparius*	Emergence and survival	Static, spike water	28 days	NOEC	30 (n)	Putt, 2006
ATSA							
Acute toxicity to fish OECD Guideline 203, GLP, purity 99%)	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Static	96 hours	LC_{50}	>119 (mm)	Marino *et al*, 2006a
Daphnia sp Acute Immobilisation OECD Guideline, 202, GLP, purity 100%)	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	>121 (mm)	Marino *et al*, 2006b
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity 100%)	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	72 hours	ErC_{50} NOErC	42.8 (mm) <3.06 (mm)	Hancock *et al*, 2006a
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity 99%)	*Lemna gibba*	Growth	Semi-static	7 days	ErC_{50} NOErC	>120 (mm) 120 (mm)	Hancock *et al*, 2006b

Pyridine sulfinic acid							
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity 98%)	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	72 hours	ErC_{50} NOErC	>97 (mm) 55 (mm)	Hoberg, 2005c
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity 98%)	*Lemna gibba*	Growth	Semi-static	7 days	ErC_{50} NOErC	>110 (mm) 110 (mm)	Hoberg, 2005e
5-OH-XDE-742							
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity 100%)	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	72 hours	ErC_{50} NOErC	>80 (mm) 80 (mm)	Hoberg, 2006a
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity 100%)	*Lemna gibba*	Growth	Semi-static	7 days	ErC_{50} NOErC	7.4 (mm) 1.7 (mm)	Hoberg, 2005f
6-Cl-7-OH-XDE-742							
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity 99%)	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	72 hours	ErC_{50} NOErC	85 (mm) 39 (mm)	Hoberg, 2006c
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity 99%)	*Lemna gibba*	Growth	Semi-static	7 days	ErC_{50} NOErC	46 (mm) 16 (mm)	Hoberg, 2005h
ADTP							
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity 98%)	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	72 hours	ErC_{50} NOErC	>92 (mm) 92 (mm)	Hoberg, 2006d
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity 98%)	*Lemna gibba*	Growth	Semi-static	7 days	ErC_{50} NOErC	>93 (mm) 93 (mm)	Hoberg, 2006i
5,7-di-OH-XDE-742							
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity 98%)	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	72 hours	ErC_{50} NOErC	60 (mm) 36 (mm)	Hoberg, 2006b
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity 98%)	*Lemna gibba*	Growth	Semi-static	7 days	ErC_{50} NOErC	>95 (mm) 37 (mm)	Hoberg, 2006g

Pyridine sulfonamide							
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity 96%)	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	72 hours	ErC_{50} NOErC	>114 (mm) 114 (mm)	Auferheide, 2007
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity 96%)	*Lemna gibba*	Growth	Semi-static	7 days	ErC_{50} NOErC	>114 (mm) 114 (mm)	Hicks, 2007

Notes:
mm refers to mean measured
*formerly *Selenastrum capricornutum*