Committee for Risk Assessment

RAC

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

Adopted

10 March 2016

10 March 2016

CLH-O-0000001412-86-102/F

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

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

Chemical name: **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**

The proposal was submitted by **the United Kingdom** and received by RAC on **21 May 2015.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

The United Kingdom has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **16 June 2015**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **31 July 2015**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Brendan Murray**

Co-Rapporteur, appointed by RAC: **Katalin Gruiz**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **10 March 2016** by **consensus**.

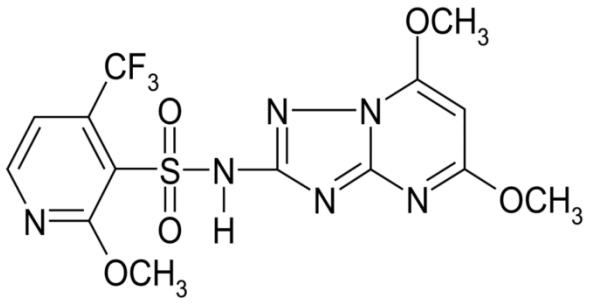
Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	**No current Annex VI entry**										
Dossier submitters proposal	TBD	pyroxsulam (ISO); *N*-(5,7-dimethoxy[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide	610-007-6	422556-08-9	Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	GHS07 GHS09 Warning	H317 H410		 M=100 M=100	
RAC opinion	TBD	pyroxsulam (ISO); *N*-(5,7-dimethoxy[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide	610-007-6	422556-08-9	Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	GHS07 GHS09 Warning	H317 H410		 M=100 M=100	
Resulting Annex VI entry if agreed by COM	TBD	pyroxsulam (ISO); *N*-(5,7-dimethoxy[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide	610-007-6	422556-08-9	Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	GHS07 GHS09 Warning	H317 H410		 M=100 M=100	

GROUNDS FOR ADOPTION OF THE OPINION

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.

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.

Assessment and comparison with the classification criteria

There is no data to indicate that classification is warranted.

HUMAN HEALTH HAZARD EVALUTATION

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.**

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.

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.

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.

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.

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**.

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 riteria 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 $\geqslant$ 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 $\leqslant$ 1% for intradermal induction with an incidence of sensitisation $\geqslant$ 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**.

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 OECD 408, GLP Strain: F344	Males: 1000 mg/kg bw/d	>1000 mg/kg bw/d	No adverse effects at highest dose tested.	Stebbins *et al* (2003)
	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	Males: 100 mg/kg bw/d	1000 mg/kg bw/d	Increased liver weight (12-18% relative - absolute)	Johnson, Books and Dryzga

Strain: CD1	Females: 1000 mg/kg bw/d	>1000 mg/kg bw/d	No adverse effects	(2003)
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**.

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).

In Vitro *studies:* 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).

In Vivo *studies:* 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**.

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

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.

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.

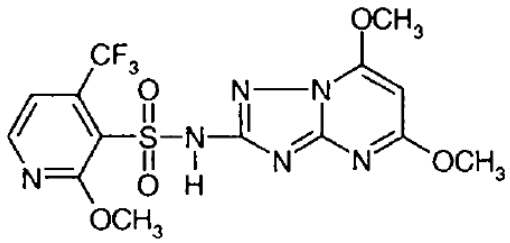
Relevance of Hepatocellular Tumours in CD-1 Mice

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:

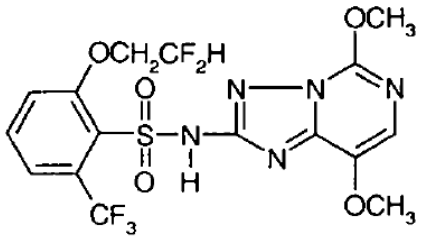
1. Liver hepatocellular tumors were observed in the CD-1 mouse. This strain of mouse has a variable and often high background incidence of this tumour type (table below).
2. The increase in tumours was specific to **males only**. This effect is also confirmed by the historical control data from Charles 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 backround level of hepatocellular tumours.

Evidence for substance-related carcinogenicity in Animals?

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.

The evidence for substance-related carcinogenicity in Animals is therefore equivocal

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.

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 statisticallysignificant 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.

Rabbit Dose-Ranging Developmental Study (Sloter, 2005a)

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.

Rabbit Developmental Study (Sloter, 2005b)

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 warranted**.

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**.

ENVIRONMENTAL HAZARD EVALUATION

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**.

Additional references

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.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

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