Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

3-(difluoromethyl)-1-methyl-N-(3',4',5'-trifluorobiphenyl-2-yl)pyrazole-4-carboxamide; fluxapyroxad

EC Number: -
CAS Number: 907204-31-3

CLH-O-0000001412-86-254/F

Adopted

30 November 2018

30 November 2018

CLH-O-0000001412-86-254/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: **3-(difluoromethyl)-1-methyl-N-(3',4',5'-trifluorobiphenyl-2-yl)pyrazole-4-carboxamide; fluxapyroxad**

EC Number: **-**

CAS Number: **907204-31-3**

The proposal was submitted by the **United Kingdom** and received by RAC on **12 December 2017.**

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 **12 March 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **11 May 2018**.

ADOPTION OF THE OPINION OF RAC

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

Co-Rapporteur, appointed by RAC: **Zilvinas Uzomeckas**

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 **30 November 2018** by **consensus**.

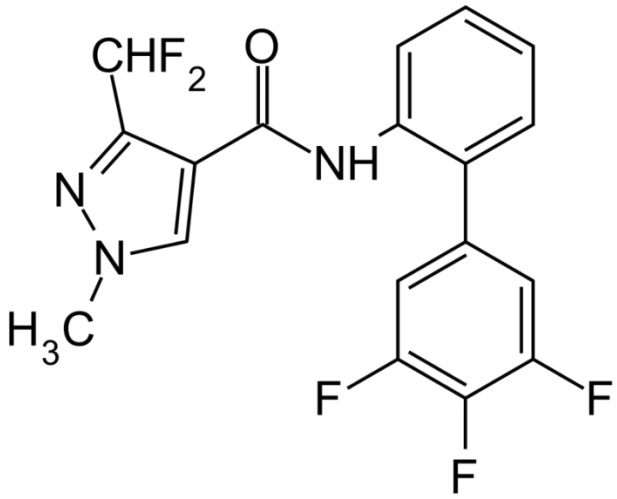
Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	3-(difluoromethyl)-1-methyl-N-(3',4',5'-trifluorobiphenyl-2-yl)pyrazole-4-carboxamide; fluxapyroxad	N/A	907204-31-3	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=1 M=1	
RAC opinion	TBD	3-(difluoromethyl)-1-methyl-N-(3',4',5'-trifluorobiphenyl-2-yl)pyrazole-4-carboxamide; fluxapyroxad	N/A	907204-31-3	Lact. Aquatic Acute 1 Aquatic Chronic 1	H362 H400 H410	GHS09 Wng	H410		M=1 M=1	
Resulting Annex VI entry if agreed by COM	TBD	3-(difluoromethyl)-1-methyl-N-(3',4',5'-trifluorobiphenyl-2-yl)pyrazole-4-carboxamide; fluxapyroxad	N/A	907204-31-3	Lact. Aquatic Acute 1 Aquatic Chronic 1	H362 H400 H410	GHS09 Wng	H362 H410		M=1 M=1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Fluxapyroxad is a pesticidal active substance in the scope of Regulation 1107/2009. It is a broad-spectrum pyrazole-carboxamide fungicide used on a large variety of commercial crops. It stunts fungus growth by inhibiting succinate dehydrogenase, the complex II in the mitochondrial respiration chain, which in turn interferes with the tricarboxylic cycle and mitochondrial electron transport. It interferes with a number of key fungal life functions, including spore germination, germ tube growth, appresoria formation and mycelium growth. It has no current entry in Annex VI of the CLP regulation and all hazard classes are open for assessment.



RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) does not propose classification of fluxapyroxad for physical hazards on the basis of the following results:

- Negative results in an EC A.14 study (*Loehr, 2008*) for testing the capability of fluxapyroxad to be explosive;
- Negative results in an EC A.10 study (*Loehr, 2008*) for testing the flammability of fluxapyroxad;
- Negative results in an EC A.13 study (*Loehr, 2008*) for testing fluxapyroxad as a pyrophoric solid;
- Data (including an EC A.16 test) indicated that no self-ignition was detected at temperatures below 400°C;
- Fluxapyroxad is not considered to be self-reactive, the SADT can be estimated to be >75°C (based on DSC measurements with an onset temperature of 290°C (energy release 30 J/g) and 335°C (energy re-lease 950 J/g) respectively;
- One EC A.12 study (*Loehr, 2008*) showed that no gas was evolved following contact of fluxapyroxad with water;
- One EC A.17 study (*Loehr, 2008*) indicated that fluxapyroxad is not oxidising.
- One ASTM G31-72 study (*Ferreira, 2009*) indicated fluxapyroxad is not corrosive to metals.

The DS also considered the following physical hazards were not applicable to fluxapyroxad (which is a solid): flammable gases, oxidizing gases, gases under pressure, flammable liquids, phyrophoric liquids, oxidizing liquids and organic peroxides.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC supports the DS's proposal for no classification of fluxapyroxad regarding physical hazards. **Classification for physical hazards is not warranted** on the basis of data obtained from several key and appropriate studies (A.14, A.10, A.13, A.16 and A.17 tests).

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of fluxapyroxad with acute oral toxicity on the basis of two negative studies performed with SD and Wistar rats according to GLP and OECD TG 423. LD_{50} > 2000 mg/kg bw in both cases. The lack of acute oral toxicity was supported by an acute neurotoxicity study performed in Wistar rats according to GLP and OECD TG 424 with no lethalities at the highest dose tested in 10 animals per sex (2000 mg/kg bw).

The DS proposed no classification of fluxapyroxad for acute dermal toxicity on the basis of no lethalities at the limit dose (2000 mg/kg bw) in a GLP and OECD TG 402 study (semi occlusive, 24-hour exposure).

The DS proposed no classification for acute inhalation toxicity. In an OECD TG 403 acute inhalation study, groups of 5 Wistar rats/sex/dose were nose-only exposed for 4 h to a dust aerosol of Fluxapyroxad at a concentration of 5.1 ± 0.33 mg/L. No mortality occurred during the study period. The particle size of the test atmosphere was 3.3 ± 2.1 µm and 3.4 ± 2.2 µm MMAD.

Comments received during public consultation

No comments were received

Assessment and comparison with the classification criteria

Acute oral toxicity

The oral LD_{50} of > 2000 mg/kg bw for rats is above the value for classification according to CLP, therefore **classification for acute oral toxicity is not warranted**.

Acute dermal toxicity

The dermal LD_{50} of > 2000 mg/kg bw for rats is above the value for classification according to CLP, therefore **classification for acute dermal toxicity is not warranted.**

Acute inhalation toxicity

The 4 h inhalation LC_{50} of > 5 mg/L for rats is above the value for classification in the CLP (i.e. 5 mg/L dust/mist). Therefore **classification for acute inhalation toxicity is not warranted**.

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

Summary of the Dossier Submitter's proposal

No significant specific target effects were observed after oral, dermal or inhalation following a single exposure event with fluxapyroxad in rats (see table 39, CLH report).

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Fluxapyroxad did not induce significant toxic effects in animal studies after single exposure and no human evidence is available, therefore classification in Category 1 or Category 2 is not warranted. Some clinical signs (increased landing foot splay, reduced raring, impaired motor activity) were considered as unspecific neuropharmacological effects resulting from bolus application of a high dose rather than being indicative of neuronal damage in the acute neurotoxicity study. Effects were fully reversible and no treatment-related neuropathological findings were observed during histopathological examination of the nervous system. Category 3 classification is warranted for substances that induce transient target organ effects after single exposure and this includes narcotic effects and respiratory tract irritation. Incidents of increased respiration or abdominal respiration from the acute inhalation study were not considered to be severe effects. Fluxapyroxad did not induce any significant irritation to mucous membranes when tested in an eye irritation study nor did it produce irritation when tested in a skin irritation study. The available data **do not support classification for STOT SE**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

In an OECD TG 404 skin irritation/corrosion study, two male and one female NZW rabbits were exposed to 0.5 g fluxapyroxad (moistened with water) under a semi-occlusive dressing for 4 h. No mortality or clinical signs of toxicity occurred. Individual mean erythema scores over 24, 48 and 72 h were 0.0, 0.0, and 0.7. Mean oedema scores were 0.0 for all animals. All signs of dermal irritation resolved within 72 h. The DS did not propose classification for skin corrosion/irritation.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

No corrosion of the skin occurred. The mean scores for erythema/eschar or oedema formation were less than the criteria (< 2.3) in all animals. Any signs of dermal irritation resolved within 72 h. The results **did not meet the criteria for classification as skin corrosive of irritant**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

In two GLP, OECD TG 405 eye irritation studies, 0.1 ml of fluxapyroxad was instilled into the conjunctival sac of the right eye of 3 NZW rabbits. Eyes were rinsed after 24 hours. The mean scores over 24, 48 and 72 h were 0.0 for corneal opacity, iris inflammation, conjunctival redness, and chemosis. The DS did not propose classification for serious eye damage/eye irritation.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

The mean individual scores of the readings 24 to 72 hours after instillation (all 0.0) do not meet the criteria for classification (average score of corneal opacity ≥ 1, and/or iritis ≥ 1, and/or conjunctival redness ≥ 2 and/or conjunctival oedema ≥ 2). Therefore **classification for serious eye damage/irritation is not warranted**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed no classification of fluxapyroxad for skin sensitisation on the basis of a GLP compliant, OECD TG 406 Guinea pig maximisation test that showed only a maximum of 10% of animals responded to the challenge during the first 24 hours (table below). This response was reduced to 5% after 48 hours. Concurrent controls had a positive response rate of 10%.

After challenge, discrete or patchy erythema (grade 1) constituting a positive response was observed in 2/20 animals (10%) at 24 hours and 1/20 animals (5%) at 48 hours compared to 1/10 in the control group following challenge with 25% fluxapyroxad. The intradermal induction concentration was 5%.

Table: *Challenge results*

Skin findings	Control group		Test group	
	24 h	48 h	24 h	48 h
Grade 0	9/10	9/10	18/20	19/20
Grade 1	-	1/10	2/20	1/20
Grade 2	1/10	-	-	-

x/y: number of animals with findings / number of animals tested

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

The Magnusson & Kligman guinea pig maximisation test with fluxapyroxad was a recent study (2008) performed according to GLP and OECD TG 406. It was described as acceptable in the DAR with no deviations from the guideline noted by the RMS. In this particular study there was no positive control (reliability check) with a known sensitizer. However, separate positive control studies were performed twice a year in the laboratory in which the study was completed. The RMS noted that the positive control with alpha-hexylcinnamaldehyde showed that the test system was validated in the performing laboratory (within 6 months of the main study, 100% positive response in an M&K maximization study). Details of individual positive control studies during the preceding 3 years (3 x M&K maximization studies and 3 x Buehler tests) were supplied as an appendix to the original study report.

No reasons were presented in the CLH report/DAR for not using higher concentrations of substance. All criteria for establishing a valid study were satisfied with the reported concentrations of fluxapyroxad.

i. For the intradermal induction treatment, the highest concentration of the test substance that causes slight to moderate irritation was determined: this was a 5% test substance preparation.

ii. For the topical induction treatment, the highest concentration of the test substance that causes slight to moderate irritation was determined: this was a 60% test substance preparation.

iii. For the challenge, the maximum non-irritant concentration was determined: this was a 25% test substance preparation.

Initial tests were carried out to determine these test substance concentrations. The Magnusson & Kligman grading scale for the evaluation of challenge patch test reactions was used, i.e.:

0 = no visible change

1 = discrete or patchy erythema

2 = moderate and confluent erythema

3 = intense erythema and swelling

A pre-test established the following findings:

iv. Intradermal injection 1: a 1:1 mixture (v/v) FCA: 0.9% NaCl → caused intense erythema and swelling (grade 3) at the injection sites.

v. Intradermal injection 2: a 5% test substance preparation in 1% CMC-solution in water → caused a moderate and confluent erythema in addition to swelling (grade 2)

vi. Intradermal injection 3: a 5% test substance preparation in Freund's adjuvant: 0.9% NaCl → caused intense erythema and swelling (grade 3).

According to these results a 5% substance preparation in Freund's adjuvant: 0.9% NaCl caused a sufficient response to satisfy the criteria for dose selection for intradermal injection.

The guideline specifies that about 24 hours before the topical induction application, if the substance is not a skin irritant, the test area should be treated with 0.5 ml of 10% sodium lauryl sulphate (SLS) in vaseline, in order to create a local irritation. However, in this case there were very clear signs of irritation following intradermal induction and later after topical induction using a 60% preparation of active substance. Though there was no explicit statement from the original study report, there was probably no need to use SLS.

In the pre-test, upon topical application, the 60% test substance preparation caused discrete or patchy to moderate and confluent erythema in 3/3 animals 1 hr after removal of the patch, and

in 1/3 animals 24 and 48hr after removal of the patch. This concentration was considered to be suitable as the highest concentration that causes slight to moderate irritation.

In the main test, following topical induction with the 60% test substance preparation, significant signs of irritation were observed consisting of partially open incrustation in addition to moderate and confluent erythema and swelling in all test group animals.

Also in the pre-test, no skin findings were observed at the application sites with a topically applied 25% test substance preparation at either 24 or 48 hours. The 25% test substance preparation in 1% CMC solution was determined to be the maximum non-irritant concentration chosen for topical challenge in the main test.

In summary, the guinea pig maximisation test conforms to the guideline. The tested concentrations appeared to satisfy the guideline with regard to intradermal/topical testing dose. The RAC considers that the study was well conducted and the 5-10% of animals showing positive reactions matches the 10% rate seen in the concurrent controls. The RAC concurs with the DS that the criteria for classification are not met and there is sufficient data to conclude on this endpoint.

A response in at least 30% of the animals in an adjuvant test is required for classification. A 5-10% response was noted in this study after a challenge with fluxapyroxad. Concurrent controls had a 10% positive response rate. The procedure was well validated in the performing laboratory with positive control studies every six months. As the criteria are not met, **classification of fluxapyroxad as a skin sensitizer is not warranted**.

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

Summary of the Dossier Submitter's proposal

The DS did not propose classification for STOT RE. The DS considered data from all studies at effect levels at or below the cut-off criteria for STOT RE 2 and described a variety of effects on haematological and clinical chemistry parameters, organ weights and histopathology of the main target organ (the liver) in rats, mice and dogs. Table 40 in the CLH report summarises the repeat dose studies on fluxapyroxad which were conducted in rats (28-day dietary, 28-day dermal, 90-day dietary, 90-day neurotoxicity (dietary)), mice (28-day dietary, 28-day immunotoxicity (dietary), 90-day dietary) and dogs (28-day dietary, 90-day dietary, 1-year dietary). Additional information regarding repeated exposure toxicity was also considered from a 2-generation toxicity study in rats and carcinogenicity studies in both rats and mice.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Table: *Summary of most relevant effects for consideration of STOT RE occurring within the trigger dose ranges.*

Study	Relevant effect level	Cat. 1 mg/kg bw/day	Cat. 2 mg/kg bw/day	Significant & Potentially Relevant Effects (dose response? Y/N)	Reference
Rat, 28-day oral dietary	2000 ppm **Males**: 176 mg/kg bw/day **Females**: 183 mg/kg bw/day	≤ 30	≤ 300	**Males**: (control vs 2000 ppm) ↑ TSH (µg/l): 6.57 vs 11.09* (Y) ↑ Abs. liver wt. (+52%)** (Y) ↑ Rel. liver wt. (+52%)** (Y) Liver: ↑ hypertrophy: 0 vs 5 (4.4)[1] (Y) Thyroid: ↑ follicular hypertrophy / hyperplasia: 1 (1.0) vs 5 (1.4)[1] (Y) **Females**: (control vs 2000 ppm) ↑ Abs. liver wt. (+37%)** (Y) ↑ Rel. liver wt. (+35%)** (Y) Liver: ↑ hypertrophy: 0 vs 5 (2.4)[1] (Y)	DAR B.6.3.1 (IIA 5.3.1/1) Note: absence of an effect on thyroid weight.
Rat, 90-day oral dietary	500 ppm **Males**: 31 mg/kg bw/day **Females**: 35 mg/kg bw/day	≤ 10	≤ 100	**Males**: (control vs 500 ppm) ↑ Abs. liver wt. (+15%)** (Y) ↑ Rel. liver wt. (+18%)** (Y) Liver: ↑ hypertrophy: 0 vs 9 (1.0)[2] (Y) **Females**: (control vs 500 ppm) ↓ Prothrombin time (s): 33.1 vs 30.6** (Y) ↑ Abs. liver wt. (+13%)** (Y) ↑ Rel. liver wt. (+13%)** (Y) Liver: ↑ hypertrophy: 0 vs 9 (1.0)[2] (Y) Thyroid: ↑ follicular hypertrophy / hyperplasia: 0 vs 4 (1.0)[2] (Y)	DAR B.6.3.1 (IIA 5.3.2/1)
Mouse, 28-day oral dietary	500 ppm **Males**: 112 mg/kg bw/day **Females**: 150 mg/kg bw/day	≤ 30	≤ 300	**Males**: (control vs 500 ppm) ↑ Abs. liver wt. (+12%)** (Y) ↑ Rel. liver wt. (+9%)** (Y) No histopathological findings. **Females**: (control vs 500 ppm) No effects	DAR B.6.3.2 (IIA 5.3.1/3)
Mouse, 90-day oral dietary	400 ppm **Males**: 77 mg/kg bw/day	≤ 10	≤ 100	**Males**: (control vs 400 ppm) Minor clinical chemistry changes (↓ triglyceride and cholesterol)	DAR B.6.3.2 (IIA 5.3.2/2)
Dog, 28-day oral dietary	7500 ppm **Males**: 211 mg/kg bw/day	≤ 30	≤ 300	**Males**: (control vs 7500 ppm) ↑ ALP (µkat/l): 1.75 vs 7.81** (Y) ↑ Abs. liver wt. (+26%) (Y) ↑ Rel. liver wt. (+27%)* (Y) (No histopathological findings)	DAR B.6.3.3 (IIA 5.3.1/4)

	Females: 230 mg/kg bw/day			**Females**: (control vs 7500 ppm) ↑ ALP (µkat/l): 1.88 vs 6.94** (Y) ↓ Abs. thymus wt. (-52%)* (Y) ↓ Rel. thymus wt. (-52%)** (Y) (No histopathological findings)	
Dog, 90-day oral dietary	1500 ppm **Males**: 45 mg/kg bw/day **Females**: 51 mg/kg bw/day	≤ 10	≤ 100	**Males**: (control vs 1500 ppm) ↑ Abs. liver wt. (+16%) (Y) ↑ Rel. liver wt. (+10%) (Y) (No histopathological findings) **Females**: (control vs 1500 ppm) Minor clinical chemistry changes (No histopathological findings)	DAR B.6.3.3 (IIA 5.3.2/3)
Dog, 12-month oral dietary	300 ppm **Males**: 8 mg/kg bw/day **Females**: 9 mg/kg bw/day	≤ 2.5	≤ 25	**Males**: (control vs 300 ppm) Minor clinical chemistry changes **Females**: (control vs 300 ppm) Minor clinical chemistry changes	DAR B.6.3.3 (IIA 5.3.2/4)
Rat, 28-day dermal	300 mg/kg bw/day	≤ 60	≤ 600	**Males**: (control vs 300 mg/kg/d) No effects. **Females**: (control vs 300 mg/kg/d) No effects.	DAR B.6.3.4 (IIA 5.3.3/1)
2-year dietary study in rats	250 ppm **Males**: 11 mg/kg bw/day	≤ 1.25	≤ 12.5	**Males**: (control vs 250 ppm) Minor clinical chemistry changes ↑ Abs. liver wt. (+11%)** (Y) Liver: ↑ hypertrophy: 1 vs 30** (animal incidence) (Y) Femur: ↑ deposition of Perl's Prussian blue positive material: 0 vs 35 (Y)	DAR B.6.5.1 (IIA 5.5.2/1)
18-month dietary study in mice	150 ppm (lowest dose tested) **Males**: 21 mg/kg bw/day **Females**: 33 mg/kg bw/day	≤ 1.67	≤ 16.7	**Males**: No relevant dose to compare with criteria. **Females**: No relevant dose to compare with criteria.	DAR B.6.5.2 (IIA 5.5.3/1)

Rat, 28-day oral dietary, Thyroid hormone study	3000 ppm **Males**: 214 mg/kg bw/day **Females**: 237 mg/kg bw/day	≤ 30	≤ 300	**Males**: (control vs 3000 ppm) ↑ Abs. liver wt. (+50%)** (Y) ↑ Rel. liver wt. (+54%)** (Y) ↑ Abs. thyroid wt. (+24%)** (Y) ↑ Rel. thyroid wt. (+33%)** (Y) (no histopathology conducted) **Females**: (control vs 3000 ppm) ↑ Abs. liver wt. (+49%)** (Y) ↑ Rel. liver wt. (+50%)** (Y) ↑ Abs. thyroid wt. (+5%) (Y) ↑ Rel. thyroid wt. (+11%) (Y) (no histopathology conducted)	DAR B.6.5.3 (IIA 5.5.4/2)
Rat, 90-day oral dietary, neurotoxicity	1000 ppm **Males**: 58 mg/kg bw/day **Females**: 67 mg/kg bw/day	≤ 10	≤ 100	**Males**: (control vs 1000 ppm) ↑ Abs. liver wt. (+35%)** (Y) ↑ Rel. liver wt. (+27%)* (Y) ↑ Abs. thyroid wt. (+42%)* (Y) ↑ Rel. thyroid wt. (+40%) (Y) Liver: ↑ hypertrophy: 0/5 vs 5/5 (animal incidence) (Y, severity) **Females**: (control vs 1000 ppm) ↑ Abs. liver wt. (+24%)** (Y) ↑ Rel. liver wt. (+24%)** (Y) ↑ Abs. thyroid wt. (+46%)* (Y) ↑ Rel. thyroid wt. (+60%)** (Y) Liver: ↑ hypertrophy: 0/5 vs 5/5 (animal incidence) (Y, severity)	DAR B.6.7.1 (IIA 5.7.4/1)
Mouse, 28-day oral dietary, immunotoxicity study	500 ppm **Males**: 106 mg/kg bw/day	≤ 30	≤ 300	**Males**: (control vs 500 mg/kg/d) No effects (limited parameters measured).	DAR B.6.8.2 (IIA 5.10/1)
Rat, 2-gen oral dietary, 70-days+	F0/F1: 1000 ppm **Males**: 50 mg/kg bw/day **Females**: 50 mg/kg bw/day	≤ 13	≤ 128	**Males**: (control vs 1000 ppm) ↑ Abs. liver wt. (+23/25%)* (Y) ↑ Rel. liver wt. (+25/28%)* (Y) ↑ Abs. thyroid wt. (+ns/14%)* (Y) ↑ Rel. thyroid wt. (+ns/17%) (Y) Liver: ↑ hypertrophy: 0/25 vs 25/25*** (animal incidence) (Y, severity) Liver: ↑ Fatty cytoplasmic vacuolation: 3-4/25 vs 18-14/25*** (animal incidence) (N) Thyroid: ↑ follicular hypertrophy / hyperplasia: 0/25 vs 25/25*** (1.2) (Y, severity)	DAR B.6.6.1 (IIA 5.6.1/1)

				Females: (control vs 1000 ppm) ↑ Abs. liver wt. (+10/9%)* (Y) ↑ Rel. liver wt. (+19/14%)* (Y) ↑ Rel. thyroid wt. (+23/ns%)* (Y) Liver: ↑ hypertrophy: 0/25 vs 25/25*** (animal incidence) (Y, severity) Liver: ↑ Fatty cytoplasmic vacuolation: 2-2/25 vs 8-9/25* (animal incidence) (N) Thyroid: ↑ follicular hypertrophy / hyperplasia: 0/25 vs 21/25*** (1.2) (Y, severity)	

* significantly different from control, $p \leq 0.05$

** significantly different from control, $p \leq 0.01$

1. animals affected out of 5 (severity grading, on a scale of 1 [minimal] to 5 [most severe])

2. animals affected out of 10 (severity grading, on a scale of 1 [minimal] to 5 [most severe])

The table above presents the most pertinent data for consideration of STOT RE classification. In all studies some effects occurred at doses within the guidance value range for STOT RE 2. It is clear that the target organ is the liver in all species and thyroid in the rat. Table 40 in the Background Document (Annex 1) provides a greater level of detail for all effects at all dose levels.

The thyroid effects (increased organ weight, hypertrophy, hyperplasia) are secondary to induction of a specific isoform of glucuronyltransferase (T4-UDP-GT) responsible for hepatic clearance of thyroid hormones. This results in elevated TSH levels and subsequent thyroid follicular cell hypertrophy and hyperplasia. However, it is noted there are no studies in bile duct cannulated animals to confirm any increase in biliary clearance. In a thyroid hormone study, conducted according to GLP, Wistar rats showed significant changes of thyroid hormone levels; increases of TSH in top dose males and females and a decrease of T4 levels in top dose males. This mechanism is considered not to be of relevance to humans in the context of the development of thyroid follicular cell tumours. It is of course relevant as a physiological general feedback response circuit amongst many species including humans. This is further assessed under the carcinogenicity section regarding thyroid tumours in rats.

The liver is the primary target organ for fluxapyroxad. Severe effects on haematological and clinical chemistry parameters, organ weights and histopathology are noted at doses above the guidance value range for STOT RE. The only significant effects that occur within the guidance value range for STOT RE 2 are the increase in liver weight accompanied by hepatocellular hypertrophy. There was no indication of hepatic single cell necrosis from any repeat study (including carcinogenicity and 2-generation rat studies) at dose levels relevant for STOT RE. There were some data for hepatocellular necrosis at high doses only (90-day study on rat, 2-generation study on rat).

The changes observed within the guidance value range for STOT RE 2 may be regarded as adaptive and do not represent significant adverse toxicological effects. Other minor changes in clinical chemistry were noted but they are not considered to indicate significant organ toxicity. Liver weight changes alone (significant and in some cases they may be considered adverse due to the magnitude of weight increase) are not considered to fulfil the criteria for classification and therefore RAC concludes that **no classification for STOT RE is warranted for fluxapyroxad**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS reported that fluxapyroxad was tested in three *in vitro* studies and two *in vivo* studies. In the CLH report, each specific study is summarised in tables 22 and 23, section 10.8. According to the DS, fluxapyroxad did not present a genotoxic hazard either in *in vitro* or *in vivo* studies. There were no studies in germ cells (justified based on the negative effects of fluxapyroxad in *in-vitro* studies and *in-vivo* mutagenicity studies in somatic cells). The DS did propose not to classify fluxapyroxad as mutagenic.

Results – *In Vitro* Tests

The bacterial (Ames) and mammalian gene mutation assays (HPRT in Chinese Hamster ovary K1 cells) resulted in negative outcomes in the presence and absence of S9. Similarly, the *in-vitro* chromosome aberration assay in Chinese Hamster V79 cells did not provide evidence of clastogenic or aneugenic activity for fluxapyroxad.

Results – In Vivo Tests

Two *in-vivo* studies investigated the potential activity of fluxapyroxad to induce micronuclei (i.e. chromosome damage) or DNA repair. No evidence of micronucleus formation was observed in male mice after duplicate administration of up to 2000 mg/kg bw within 24 hours. In a preliminary toxicity screening no difference between male and female mice was observed. Thus only male mice were tested. No decline of PCEs in the bone marrow was observed. Data from a toxicokinetic study in another rodent species (rat) indicated that fluxapyroxad and/or its metabolites reaches the blood/bone marrow.

Negative results were obtained in all studies with fluxapyroxad. There is no evidence of genotoxicity for this substance.

Table: *Summary of genotoxicity tests with fluxapyroxad adapted from table 22 in the CLH report.*

Study	Result	Test System	Reference
***In vitro* studies:**			
Bacterial mutagenicity	negative	GLP, OECD TG 471 (1997) *Salmonella* Strains: TA1535, TA1537, TA98, TA100 *E. coli* WP2 uvrA$^-$	Schulz & Landsiedel (2008e)
Mammalian cell mutagenicity	negative	GLP, OECD TG 476 (1997) CHO-K1 (HPGRT locus)	Schulz & Landsiedel (2007a)
Clastogenicity	negative	GLP, OECD TG 473 (1997) Chinese Hamster V79 cells	Schulz & Landsiedel (2008b)
***In vivo* studies:**			
Micronucleus	negative	GLP, OECD TG 474 (1997) Male NMRI mouse bone marrow (short term)	Anonymous (2006b)
UDS	negative	GLP, OECD TG 486 (1997) Wistar rat, male (single oral gavage); hepatocytes	Anonymous (2008g), Amendment: Anonymous (2008h)

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

No human data are available for fluxapyroxad, therefore a classification with Muta. 1A is not supported. Fluxapyroxad is negative in acceptable *in vitro* tests and *in vivo* somatic cell mutagenicity guideline tests in mammals. Data are not available illustrating the induction of mutagenic effects in germ cells (a criterion for Category 1B).

There was no evidence of fluxapyroxad genotoxicity in somatic cells *in-vivo* or for *in-vitro* genotoxicity and therefore no classification in category 2 is warranted.

RAC agrees with the DS that **there is no evidence to support classification of fluxapyroxad for genotoxicity**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Two guideline and GLP compliant long-term oral (dietary) toxicity/carcinogenicity studies were available to the DS: a 2-year combined chronic toxicity/carcinogenicity study in the Han Wistar rat (*Anonymous, 2009d*) and an 18-month carcinogenicity study in the C57BL/6J Rj mouse (*Anonymous, 2009e/amendment 2010a*). Study details were summarised in Table 24 in the CLH report. Fluxapyroxad induced liver and thyroid tumours in rats. Several additional studies were conducted to investigate the Mode of Action (MoA) and human health relevance of the rodent tumours. The DS concluded from this data that the observed tumours were not considered to be of relevance to humans and consequently, no classification for carcinogenicity was proposed.

In-vivo animal studies

Rat 2-year dietary toxicity/oncogenicity study

In the rat carcinogenicity study (*Anonymous, 2009d*) treatment with fluxapyroxad did not affect the survival of rats up to the highest dose. Crl:WI (Han) strain rats were divided into treatment groups and scheduled kills were conducted after 12 months treatment for 10 animals/sex/group and at study termination after 24 months treatment for 50 animals/sex/group.

***Table 4**: Mean dose received (mg/kg bw/day)*

Dietary concentration of fluxapyproxad (ppm)	50	250	1500	3000
Males	2.1	11	68	145
Females	2.7	14	82	182

General toxicity displayed by reduced body weight and body weight gain was observed in males at 145 mg/kg bw/day and in females at ≥ 14 mg/kg bw/day. The liver, thyroid, bone and haematological system were identified as targets in the rat. The effects observed included increased liver weight and hepatocellular hypertrophy, spongiosis hepatis (cystic degeneration), thyroid follicular cell hyperplasia, iron disposition in the femur, hyperostosis of skull bones, tooth whitening, accelerated clotting and reduced MCH and MCV.

Organ weight changes considered to be treatment-related were observed in the liver and thyroid. There were no treatment-related neoplastic findings at the 12-month interim sacrifice.

Liver Tumours

Adenomas: There were significantly increased incidences of hepatocellular adenomas in males at ≥ 11 mg/kg/day and in females at ≥ 82 mg/kg/day. The incidence was 0, 0, 4 (8%), 7 (14%), 15 (30%) in males and 0, 2 (4%), 0, 4 (8%) and 7 (14%) in females in the controls and increasing dose groups, respectively. A clear dose response was evident. The laboratory historical control range[1] of hepatocellular adenomas was 0-4% in males and 0-6% in females.

Carcinomas: In males there was also a significantly increased incidence of hepatocellular carcinomas at the highest dose of 145 mg/kg bw/day. The incidence was 0, 1 (2%), 0, 3 (6%), 9 (18%) in the controls and increasing dose groups, respectively; the laboratory historical control range[1] of hepatocellular carcinomas in males was 0-6%. The combined incidence of hepatocellular tumour bearing animals was 1, 0, 5, 10 and 21 in males and 1, 3, 0, 4 and 7 in females.

Table: *Liver tumour findings in animals scheduled for termination at 24 months: number of animals affected*

Parameter	Dose of fluxapyroxad (mg/kg bw/day)									
	Males					Females				
	0	**2.1**	**11**	**68**	**145**	**0**	**2.7**	**14**	**82**	**182**
Liver										
no. exam.	50	50	50	50	50	50	50	50	50	50
- adenomas	0	0	**4**	**7****	**15****	0	2	0	**4**	**7****
- carcinomas	1	0	1	3	**9****	1	1	0	0	0
- combined tumours	1	0	**5**	**10****	**21**[1]	1	3	0	**4**	**7***

[1] 3 animals with adenoma and carcinoma

*significantly different from control, p ≤ 0.05

** significantly different from control, p ≤ 0.01

Thyroid Tumours

A slight increase in thyroid follicular cell adenomas and carcinomas was observed in males at 68 mg/kg bw/day and 145 mg/kg bw/day. The incidence of carcinomas at the highest dose was slightly above the laboratory historical control range (3 out of 50 or 6% at 145 mg/kg bw/day vs. the historical range[1] of 0-4%, mean of 2.3%) indicating a possible relationship with fluxapyroxad treatment. However, the incidence of adenoma was well within the laboratory historical control range (9 animals or 18% at the two highest doses vs. the historical range[1] of 4-28% and mean of 13%) which indicated that a relationship with treatment was unlikely. The incidence of follicular cell tumours in females was not affected by treatment.

[1] historical control range based on observations from 400 control Crl:WI(Han) strain rats in studies conducted at BASF SE from 1999 to 2008

All other neoplastic findings occurred in single or low incidences or were evenly distributed between all groups, including the controls and were therefore considered to be of spontaneous origin.

Table: *Thyroid tumour findings in animals scheduled for termination at 24 months: number of animals affected*

Parameter	Dose of fluxapyroxad (mg/kg bw/day)									
	Males					Females				
	0	2.1	11	68	145	0	2.7	14	82	182
Thyroid glands no. exam.	50	50	50	50	50	50	49	50	48	50
- Adenoma, C-cell	5	6	4	2	6	13	6	6	5	8
- Carcinoma, C-cell	1	0	0	0	1	0	1	0	0	0
- Adenoma, follicular cell	3	2	4	**8**	**9**	0	3	1	3	2
- Carcinoma, follicular cell	0	0	1	1	3	2	0	1	0	1
- combined follicular cell tumours	3	2	5	9	**11***[2]	2	3	2	3	3

[2] 1 animal with adenoma and carcinoma

*significantly different from control, $p \leq 0.05$

Mouse 18-month dietary toxicity/oncogenicity study

In the mouse carcinogenicity study (*Anonymous, 2009e/2010a*) treatment with fluxapyroxad did not affect the survival of mice up to the highest dose. C57BL/6J Rj strain mice were divided into treatment groups and scheduled kills were conducted after 9 months treatment for 10 animals/sex in controls and the high dose group and at study termination after 18 months treatment for 50 animals/sex/group.

Table: *Mean dose received (mg/kg bw/day)*

Dietary concentration of fluxapyproxad (ppm)	150	750	3000	6000 Killed at 9 mo.	6000 Killed at 18 mo.
Males	21	107	468	1119	996
Females	33	158	652	1512	1307

The liver was identified as the only target organ as indicated by increases in relative and/or absolute liver weights. Organ weight changes considered to be treatment related were observed in the liver both at the interim sacrifice and study termination. Increases in group mean absolute and bodyweight-related liver weights occurred in males at ≥ 21 mg/kg bw/day and in females at ≥ 652 mg/kg bw/day. Histopathological examination of the liver revealed mainly central or peripheral hepatocellular hypertrophy. In addition, an increased severity and/or incidence of macrovesicular fatty change in hepatocytes was observed at dose levels ≥ 750 ppm. There were no neoplastic findings in the animals killed at 9 months.

Liver Tumours

In animals sacrificed at 18 months, the overall incidence of neoplastic findings in the treated groups was like that in the controls. The incidence of liver tumours in fluxapyproxad treated

males was greater than controls, but a dose response relationship was not present. Among the females, 2 (4%) at 652 mg/kg bw/day and 3(6%) at 1307 mg/kg bw/day had liver adenomas; these incidences were within the background range for the C57BL strain mice (historical range of 0-6% and mean of 0.9%; 8 studies conducted between 1999 and 2008).

Table: *Neoplastic findings in the livers of animals at study termination (18 months): number of animals affected*.

Parameter	Dietary concentration of fluxapyproxad (mg/kg bw/day)									
	Males					Females				
	0	21	107	468	996	0	33	158	652	1307
Liver										
no. exam.	50	50	50	50	50	50	50	50	50	50
- Adenoma	0	3	1	1	2	0	0	0	2	3
- Carcinoma	1	3	1	3	3	1	0	0	0	0
- combined tumours	1	5[#]	2	4	5	1	0	0	2	3
- Hemangioma	0	0	1	0	0	1	0	0	0	0
- Hemangiosarcoma				1						

[#] 1 animal with adenoma and carcinoma

The incidence of hepatocellular adenoma appeared to be higher than in controls (in females). However, it did not display a dose-response relationship in males and it was within the historical control range in females. These changes were considered not treatment-related by the DS. In conclusion, the mouse carcinogenicity study did not provide robust evidence for carcinogenic potential.

Mechanism of action and supporting data relevant for findings in the rat liver

Description and results from the mechanistic studies

The DS described in detail several mechanistic studies that were designed to address the weak carcinogenic response observed in rats. A number of possible mechanistic explanations were considered and the available investigations focused on a non-genotoxic mode of action involving hepatocyte proliferation, induced via constitutive androstane receptor (CAR) and/or pregnane X receptor (PXR) activation.

The DS summarised the available studies which included:

- 1 x *in-vitro* rat microsome study on enzyme inhibition (table 25, CLH report).
- 3 x *in-vitro* studies with rat hepatocytes.
- 1 x *in-vitro* study with human hepatocytes.
- 3 x *in-vivo* rat hepatocellular proliferation studies.
- 1 x *in-vivo* 14-day dietary rat enzyme induction study with 28-day recovery

There were no *in-vivo* studies with CAR-knock out rats. The *in-vitro* rat hepatocyte studies included hepatocytes from both wild-type and CAR-KO rats and investigated effects on expression signatures for CAR, PXR, AhR and PPARα. The in-vitro human hepatocyte study included hepatocytes derived from two donors with the results reported individually per donor. Donor 2 was significantly compromised (a 39 year old Caucasian male, suffering from multiple sclerosis with multiple medical drug treatments and positive urine test for drugs including THC, opiates and cocaine). The 14-day dietary rat enzyme inhibition study showed liver effects consistent with a typical CAR activator; a dose-dependent increase of total cytochrome P450 content (max. 2.1x), EROD (max. 3.1x), PROD (max. 20x) in males, max. 125x in females), and BROD (max. 10x in males, max. 127x in females) was observed. All effects were reversible within the 28-day recovery period.

The mechanistic studies showed that fluxapyroxad increased gene transcription and activity of Phase I and Phase II xenobiotic metabolising enzymes in the livers of rats in a pattern that is consistent with activation of CAR/PXR nuclear receptors. A similar induction profile was also seen in cultured human hepatocytes. Although fluxapyroxad clearly had the potential to induce hepatocellular proliferation in rats, it did not induce proliferation in cultured human hepatocytes. AhR involvement was shown to be minimal, fluxapyroxad could not be described as a prototypical AhR receptor agonist and there was no indication of PPARα activation.

Inhibition of apoptosis and other associative events in the CAR-associated tumour model have not been investigated (e.g. altered epigenetic changes, gap junctional intercellular communication and oxidative stress). The available mechanistic data do indicate that the MoA for liver tumours in rats is secondary to hepatocellular proliferation induced by activation of the CAR.

Conclusions

The experiments in hepatocytes from CAR knockout (KO) Sprague-Dawley rats demonstrate the crucial role of the nuclear receptor CAR as essential key events are no longer observed in the absence of this receptor, i.e. the alteration of gene expression specific to CAR and most importantly the lack of hepatocellular proliferation. The similarity of effects in wild type (WT) Wistar and Sprague-Dawley hepatocytes confirm that the mechanistic information obtained *in-vitro* in WT and CAR KO hepatocytes from Sprague-Dawley rats are relevant for the *in-vivo* situation in Wistar rats.

Information from comparison studies conducted using donor human hepatocytes and hepatocytes from CAR knockout rats indicate that there are clear species differences between rats and humans. In particular human hepatocytes lack the capacity to mount a proliferative response to CAR activation, and progression to liver tumours in humans via CAR activation is considered unlikely. The DS did not consider the rat liver tumours (induced by fluxapyroxad and arising via CAR activation) to be relevant for human.

Mechanism of action and supporting data relevant for findings in the rat thyroid

Description and results from the mechanistic studies

The DS also described a series of studies that were conducted to investigate the MoA for induction of thyroid follicular tumours. A slight increase in thyroid follicular cell adenomas and carcinomas was observed in one sex, and one species only (male rats) at ≥ 68 mg/kg bw/day. Only the carcinomas were slightly outside of the historical control data. The available studies investigated:

- Phase I and II enzyme induction in the liver, specifically those enzymes known to be involved in metabolism of thyroid hormones,
- direct action of fluxapyroxad on the thyroid (thyroid peroxidase)
- early changes in thyroid hormone levels.

The mechanistic studies showed that fluxapyroxad induced a specific isoform of glucuronyltransferase (T4-UDP-GT) which is typically responsible for hepatic clearance of thyroid hormones. However, there are no studies in bile duct cannulated animals to confirm an actual increase in biliary clearance. The negative findings for fluxapyroxad in the perchlorate discharge test preclude a direct effect on the thyroid via TPO inhibition. The *in-vivo* enzyme induction studies show thyroid follicular hypertrophy/hyperplasia and increased TSH suggesting a perturbation to the rat pituitary-thyroid axis. The repeated dose thyroid hormone study was variable in the effects noted for males and females. Overall indications from the study were however in general agreement with a perturbation to the pituitary-thyroid axis, e.g. significant

changes of thyroid hormone levels were restricted to increases of TSH in top dose males, (females were too variable); decreased T4 in males only (high dose), no change in T3; increased thyroid weight, males only (high dose) and all accompanied by an increase in liver weight.

Other potential MoA such as the inhibition of the thyroid Na^+ /I^- symporter or inhibition of type I or type II deiodinases have not been investigated.

Conclusions

The mechanistic experiments suggest fluxapyroxad induced a specific isoform of glucuronyltransferase (T4-UDP-GT) responsible for hepatic clearance of thyroid hormones. The DS believes that the most plausible interpretation of the available data is CAR/PXR induction of T4-UDP-GT with elevated TSH levels, thyroid follicular cell hyperplasia and eventual progression to follicular cell tumours. The DS considers that the observed thyroid tumours in the rat are not of relevance to human hazard assessment and proposes no classification for carcinogenicity.

Comments received during public consultation

One Member State commented. They were not convinced that a carcinogenic affect in humans could be ruled out and were concerned with the increase in CYP1A mRNA expression and EROD activity.

Assessment and comparison with the classification criteria

Introduction

Fluxapyroxad induced liver and thyroid tumours in rats and thus there is a need to consider whether classification for carcinogenicity is appropriate. There is no information from studies in humans to inform on carcinogenic potential and so classification in category 1A can be excluded.

Rat Liver Tumours

Fluxapyroxad induced liver tumours in male and female rats, with the tumour incidence in males being much greater than in females. A clear dose response was evident for both sexes. In males the incidences for both adenomas and carcinomas were above the historical control data. There was no indication from interim sacrifice data that tumour latency was reduced. No increases in liver tumours were noted in the mouse lifetime study.

There are various possible mechanistic explanations that can be considered for this carcinogenic response in rats and a limited investigation into these other modes of action was undertaken:

- genotoxicity → negative data in this case → conclusion: unlikely
- cytotoxicity → the liver is the target organ but no data to support this as a primary MoA.
- PPARα receptor activation → negative results in this case → conclusion: unlikely
- CAR/PXR receptor activation → positive data in this case → conclusion: plausible
- AhR receptor activation → limited data → conclusion: unlikely
- Porphyria → no data
- Endocrine mediated proliferation → no data, no evidence from other studies.
- Immunosuppression → limited data → no evidence of immunotoxicity in a male mouse 28-day dietary immunotoxicty study where doses up to 1323 mg/kg bw/day were tested (no substance-related effect on spleen and thymus weights, lymphocyte count

and subpopulation distribution, primary humoral (IgM response) immune response to SRBC and natural killer cell activity; DAR section B.6.8.2).

Recognising that fluxapyroxad may be associated with a hepatocarcinogenic effect in rats, the applicant sponsored a series of mechanistic studies to investigate a possible non-genotoxic mode of action involving liver stimulation via constitutive androstane receptor (CAR) and pregnane X receptor (PXR) induction. The DS presented these studies and others from the plant protection product DAR. The key events in this process are considered to be:

- CAR activation
- Altered gene expression specific to CAR activation
- Increased cell proliferation
- Inhibition of apoptosis
- Clonal expansion leading to altered foci
- Liver adenomas/carcinomas

Such a non-genotoxic mode of action has been considered of limited relevance to humans as the initial key events of this MoA can also occur in humans. However, the liver tumours resulting from the CAR(/PXR)-mediated MoA may be of little to no relevance to humans, given the difference observed in the prerequisite step for tumour formation, i.e. no DNA replication (and no increased cell proliferation) upon treatment of human hepatocytes with fluxapyroxad.

The mechanistic studies showed the following (see qualitative summaries in table below):

1. Fluxapyroxad increased rat hepatocyte gene transcription and activity of Phase I and Phase II xenobiotic metabolising enzymes consistent with activation of CAR/PXR nuclear receptors.
2. Fluxapyroxad markedly reduced the expression of CYP2B and PROD and BROD enzyme activity in CAR-KO rat hepatocytes.
3. Liver weight increased with concomitant hepatocellular hypertrophy.
4. Fluxapyroxad increased replicative DNA synthesis in a PB-like manner in rat wild type hepatocytes.
5. Fluxapyroxad did not increase replicative DNA synthesis in rat CAR-KO hepatocytes.
6. Fluxapyroxad did not increase replicative DNA synthesis in human hepatocytes.

Table: *RAC Summary of mode of action studies investigating liver tumours*

Endpoints investigated	Summary observations	Reference
1. In-vitro rat microsomes - Liver enzyme inhibition - PROD & BQ - Table 25 CLH report - Sex: Not specified.	1. Fluxapyroxad inhibited CYP2B enzyme activity (PROD) 2. Fluxapyroxad did not inhibit CYP3A enzyme activity (BQ)	Anonymous (2016)
2. In-vitro rat hepatocytes - Sprague-Dawley strain - Sex: Male - wild type animals - CAR knock-out animals - 3-day treatment - Flux, PB, EGF tested - Table 26 CLH report	**a. Cytotoxicity:** 1. ↓ ATP at 300 µM fluxapyroxad **b. Enzyme induction:** 1. Fluxapyroxad induces CYP2B and CYP3A enzyme activity indicative of CAR and PXR activation.	Elcombe (2016a)

	2. CAR KO rat hepatocytes → substantially reduced CYP2B enzyme activity **c. Replicative DNA Synthesis:** 1. Fluxapyroxad increases labelling index in a PB-like manner, dependent on CAR activation, positive EGF control. 2. CAR-KO hepatocytes confirm CAR dependency. Reduction of labelling index relative to controls.	
3. In-vitro rat hepatocytes - Sprague-Dawley strain - Sex: Male - wild type animals - CAR knock-out animals - 3-day treatment - Flux, PB, EGF tested - mRNA induction - low [fluxapyroxad] tested (1, 2, 4, 8 and16 µM) - Table 27 CLH report AhR Expression signature: - Enzymes: EROD - mRNA: CYP1A1/CYP1A2 PPARα Expression signature: - Enzymes: PCoA/LAH - mRNA: CYP4A1/Acox1	**a. Cytotoxicity:** 1. No effect up to 16 µM fluxapyroxad **b. Enzyme induction:** 1. Fluxapyroxad induces CYP2B and CYP3A enzyme activity indicative of CAR and PXR activation. 2. Fluxapyroxad does not activate PPARα (no increase in PCoA, LAH) 3. Fluxapyroxad is not a prototypical AhR receptor agonist 4. CAR KO rat hepatocytes → substantially reduced CYP2B enzyme activity **c. mRNA expression:** 1. Fluxapyroxad predominantly increases CYP2B mRNA expression → acts as a prototypical CAR activator similar to PB. 2. Fluxapyroxad shows little effect on AhR and PPARα mediated mRNA expression profiles. 3. CAR KO rat hepatocytes → confirmation of CAR dependency for fluxapyroxad activity. **d. Replicative DNA Synthesis:** 1. Fluxapyroxad increases labelling index in a PB-like manner, dependent on CAR activation, positive EGF control. 2. CAR-KO hepatocytes confirm CAR dependency.	Elcombe (2016c)
4. In-vitro rat hepatocytes - Wistar strain - Sex: Male - wild type animals - No CAR knock-out animals - 3-day treatment - Flux, PB, EGF tested - mRNA induction - high [fluxapyroxad] tested (1, 3, 10, 30 and 100 µM) - Table 28 CLH report	**a. Cytotoxicity:** 1. No biologically significant effect up to 100 µM fluxapyroxad. Other effects parallel those for enzyme induction activity, mRNA expression and hepatocellular proliferation as seen in wild type SD rat hepatocytes (see 3 above). Fluxapyroxad appeared to act as a prototypical CAR activator similar to PB.	Elcombe (2016d)
5. In-vitro human hepatocytes - 2 x donors, individually analysed, donor 2 unreliable. - Sex: Male. - Flux, PB, EGF tested - mRNA induction - high [fluxapyroxad] tested (1, 3, 10, 30 and 100 µM) - Table 29 CLH report	**a. Cytotoxicity:** 1. ↓ ATP by 59% at 100 µM fluxapyroxad **b. Enzyme induction:** 1. Fluxapyroxad induces CYP2B6 and CYP3A4 enzyme activity indicative of CAR and PXR activation but weaker than in rat. **c. mRNA expression:** 1. Fluxapyroxad increases both CYP2B6 and CYP3A4 mRNA expression → acts as a weak CAR/PXR activator.	Elcombe (2016e)

	d. Replicative DNA Synthesis: 1. Fluxapyroxad did not increase the labelling index. Positive EGF control.	
6. In-vivo rat hepatocellular proliferation (I) - Wistar (Crl:WI Han). - Males & Females - Treatment: 7, 28, and 91 days - Dietary study - Brd immunostaining - Table 30 CLH report	**a. 91-day treatment:** 1. Males: 183 mg/kg bw/day - S-phase response ↑ 2.8x 2. Females: ≥ 17 mg/kg bw/day - S-phase response ↑ up to 7.1x **b. 28-day treatment:** 1. Males: Not reported 2. Females: ≥ 15 mg/kg bw/day - S-phase response ↑ up to 13.7x **c. 7-day treatment:** 1. Males: ≥ 61 mg/kg bw/day - S-phase response ↑ up to 21.2x (zone 3) 2. Females: ≥ 18 mg/kg bw/day - S-phase response ↑ up to 25.6x	Anonymous (2010b)
7. In-vivo rat hepatocellular proliferation (II) - Wistar (Crl:WI Han). - Males & Females - Treatment: 7, 28, and 91 days - Dietary study - Brd immunostaining - Low [fluxapyroxad] tested (50ppm) - Table 30 CLH report	**a. 91-day treatment:** S-phase response: No treatment-related effects. Top dose tested 2.8 (M) and 3.2 (F) mg/kg/day. **b. 28-day treatment:** 1. Males: Not reported 2. Females: 3.1 mg/kg bw/day - S-phase response ↑ 1.9x **c. 7-day treatment:** S-phase response: No treatment-related effects. Top dose tested 2.5 (M) and 2.9 (F) mg/kg bw/day.	Anonymous (2010c)
8. In-vivo rat hepatocellular proliferation (III) - Wistar (Crl:WI Han). - Males & Females - Treatment: 1, 3, 7, and 14 days - Dietary study - Brd immunostaining - High [fluxapyroxad] tested - Table 30 CLH report	**a. 14-day treatment:** 1. Males: ≥ 106 mg/kg bw/day - S-phase response ↑ up to 2.0x 2. Females: ≥ 20 mg/kg bw/day - S-phase response ↑ up to 10.9x (zone 3). **b. 7-day treatment:** 1. Males: ≥ 100 mg/kg bw/day - S-phase response ↑ up to 14.8x (zone 3) 2. Females: ≥ 92 mg/kg bw/day - S-phase response ↑ up to 17.3x (zone 2) **c. 3-day treatment:** 1. Males: ≥ 93 mg/kg bw/day - S-phase response ↑ up to 10.5x (zone 3) 2. Females: ≥ 15 mg/kg bw/day - S-phase response ↑ up to 10.3x	Anonymous (2010d)
9. In-vivo rat enzyme induction study - Wistar (Crl:WI Han). - Males & Females - Treatment: 14 days, 28-day recovery - Dietary study - [fluxapyroxad] 0, 250, 1500, 3000 ppm - Hepatic enzymes - TSH, T4, T3 - Table 31 CLH report	**a. Organ effects:** 1. Abs liver wt.: ↑ at ≥ 16 mg/kg bw/day up to 52% in males and up to 44% in females; dose response, reversible. 2. Liver centrilobular hepatocellular hypertrophy: ↑ at ≥ 16 mg/kg bw/day. **b. Total CypP450:** 1. Males: ↑ up to 2x (dose response, reversible) 2. Females: ↑ up to 2x (dose response, reversible)	Anonymous (2009f)

	c. Enzyme induction: 1. Fluxapyroxad induces significant increase in PROD and BROD (with a small level of EROD) enzyme activity indicative of CAR activation.	

The available experimental data for fluxapyroxad indicate that the CAR-mediated MoA is the most likely mechanism for induction of rat liver tumours; with key mechanistic events demonstrated in the wild-type (WT) Sprague-Dawley and Wistar rats but not in CAR-KO SD rats. Studies in primary human hepatocytes demonstrated that the initial key events of the proposed CAR mediated mechanism, i.e. CAR activation and alteration of gene expression specific to CAR can also occur in human hepatocytes. However, proliferation (essential for subsequent tumour formation) is not observed in primary human hepatocytes. Therefore, it is concluded that the carcinogenicity in rats appears to proceed via CAR activation, which is a mechanism with limited relevance to humans.

Thyroid tumours

Fluxapyroxad induced a slight increase in thyroid follicular cell adenomas in male rats in a lifetime dietary study. A slightly increased number of thyroid follicular cell adenoma (3, 2, 4, 8 and 9 out of 50 animals) and carcinoma (0, 0, 1, 1 and 3) was noted in males. While the number of follicular cell adenoma (18%) at the high dose was within the historical control range (adenoma: 4 to 28%; 8 studies with 400 control males conducted between 1999 and 2008), the incidence of follicular cell carcinoma (6%) exceeded the historical control range (0 - 4%). The incidence of follicular cell tumours in females was within the historical control range and not indicative of a treatment-related effect. Thyroid tumours were not observed in mice. Effects on the thyroid were not observed in mice or dogs.

There are various possible mechanistic explanations that can be considered for this carcinogenic response in rats and a limited investigation into these other modes of action has been undertaken:

- genotoxicity → data in this case → conclusion: unlikely
- cytotoxicity → the thyroid is also a target organ but no data to support this as a primary MoA.
- Type I Deiodinase inhibition → no data
- Type II Deiodinase inhibition → no data
- TPO inhibition → negative in a perchlorate discharge test → conclusion: unlikely
- Na^+ /I^- symporter inhibition → no data
- Induction of hepatic glucuronyltransferases → positive data → conclusion: plausible
- Autoimmune disease → no data, no evidence
- Iodine deficiency → no data, no evidence

Recognising that fluxapyroxad may cause thyroid follicular tumours in rats, the applicant sponsored a series of mechanistic studies to investigate a possible non-genotoxic mode of action involving induction of hepatic glucuronyl transferases responsible for the hepatic clearance of T3 and T4. The key events in this process are considered to be:

- CAR activation
- Altered gene expression specific to CAR activation

- Induction of specific hepatic glucuronyl transferases
- Increased biliary clearance of T3 and T4
- Feedback increase in serum TSH
- Thyroid follicular proliferation
- Thyroid tumours

Such a non-genotoxic mode of action has been considered of no relevance to humans in the context of thyroid follicular cell tumour induction.

The mechanistic studies showed the following (table 10):

1. Fluxapyroxad increased rat thyroid follicular hypertrophy/hyperplasia.
2. Fluxapyroxad caused altered gene expression indicative of CAR activation.
3. There was an increase in the glucuronosyltransferase enzyme activity.
4. Male rats were more susceptible to fluxapyroxad than females and showed increased TSH with reduced levels of T4.
5. Fluxapyroxad did not affect organification of iodine (negative perchlorate discharge test).
6. Fluxapyroxad did not increase replicative DNA synthesis in human hepatocytes.

Table: *RAC Summary of mode of action studies investigating thyroid tumours*

Endpoints investigated	Summary observations	Reference
1. In-vivo rat enzyme induction study - Wistar (Crl:WI Han). - Treatment: 14 days, 28-day recovery - Dietary study - [fluxapyroxad] mg/kg/day; M: 0, 16, 96, 192 F: 0, 19, 126, 234. - Hepatic enzymes - TSH, T4, T3 - Table 31 CLH report	**a. Organ effects:** 1. Thyroid follicular hypertrophy/hyperplasia: ↑ at ≥ 16 mg/kg/day (males), dose response. **b. Enzyme induction:** 1. Fluxapyroxad induces glucuronosyltransferase enzyme activity (MUF-GT, HOBI-GT and T4-UDP-GT) indicative of CAR activation. 2. T4-UDP-GT: males: 1.1x, 1.52x**, 1.58x**; females: 1.81x, 2.38x**, 2.68x**; dose response, reversible within recovery period. **c. Thyroid hormones:** 1. Males: ↑ TSH** up to 2x (dose response, reversible) 2. No sig. changes in T3 or T4	Anonymous. (2009f)
2. In-vivo rat enzyme induction study - Wistar (Crl:WI Han). - Treatment: 14 days, 28-day recovery - Dietary study - Low [fluxapyroxad] mg/kg/day; M: 0, 3.0 F: 0, 3.8 - Hepatic enzymes - Table 32 CLH report	**a. Organ effects:** 1. Liver: ↑ wt in males, not statistically significant. 2. No effect on thyroid. **b. Enzyme induction:** 1. Low levels of fluxapyroxad in the rat caused a weak induction of BROD (in both sexes) and HOBI-GT (in males) liver enzyme activities.	Anonymous. (2010e)

3. Perchlorate Discharge test - Wistar (Crl:WI Han). - thyroid function test - Dietary study, 14-days - [fluxapyroxad] mg/kg bw/day; M: 0, 283 F: 0, 247 - Table 33 CLH report	**a. Organ effects:** 1. Perchlorate blockade did not cause a discharge of radioactive iodide from the thyroid in the fluxapyroxad and PB treated animals. 2. No evidence of a direct effect on the thyroid (e.g. by inhibiting thyroid peroxidase, TPO) regarding the organification of iodine.	Anonymous. (2009g)
4. Early thyroid hormone changes – Rat 28-day repeated dose study - Wistar (Crl:WI Han). - Males & Females - thyroid function test - Dietary study, 28 days - [fluxapyroxad] mg/kg bw/day; M: 0, 3.2, 16, 96, 192 F: 0, 3.8, 19, 126, 234 - TSH, T4, T3 at: days -3, 3, 7, 14, 21, 28 - Table 34 CLH report	1. ↑ TSH** levels in males (females variable), highest dose. 2. ↓ T4* levels in males, highest dose, days 14, 21, 28. 3. ↑ thyroid weight** in males, highest dose 4. ↑ liver weight**, both sexes. 5. Fluxapyroxad perturbs the pituitary-thyroid axis in rats and is responsible for alterations in thyroid hormone homeostasis and thyroid absolute and relative weights.	Anonymous. (2009h)

Fluxapyroxad induced a specific isoform of glucuronyltransferase (T4-UDP-GT) responsible for hepatic clearance of thyroid hormones. However, there was no data available on actual biliary elimination of T4 and T3. The overall data suggests that a prolonged oral dosing of fluxapyroxad causes TSH-mediated stimulation of the thyroid in rats, and eventually thyroid tumours. The most plausible mode of action is CAR/PXR induction of T4-UDP-GT, clearance of thyroid hormones prompting increased TSH production by the pituitary gland, thyroid follicular cell hyperplasia and eventual progression to follicular cell tumours. The T4 clearance hypothesis is somewhat inconsistent however, because in some rat studies T4 levels were not reduced in groups in which TSH levels were elevated and thyroid hypertrophy/hyperplasia occurred in groups in which a T4/T3 reduction was not detected. However, hormonal measurements are frequently equivocal. If we examine the repeated dose toxicity studies in further detail it is evident that the thyroid in rat is a target organ following fluxapyroxad treatment. Some points are worth noting in an overall weight of evidence approach that supports thyroid effects consistent with perturbations in TSH and associated thyroid histopathology:

- Thyroid tumours and effects on the gland itself are limited to rats
- 28-day rat oral dietary study:
  - ↑ thyroid hypertrophy/hyperplasia, males
  - ↑ altered colloid in thyroid, males
  - ↑ TSH, stat. sig., dose response, males
  - ↓ T4 levels, not stat. sig., males
- 90-day rat oral dietary study:
  - ↑ thyroid hypertrophy/hyperplasia, dose response, males + females
  - ↑ TSH, stat. sig., dose response, females (inconsistent in males but ↑ at high fluxapyroxad doses)
- 2-year rat oral dietary study:
  - ↑ thyroid hypertrophy/hyperplasia, males
  - ↑ altered colloid in thyroid, males + females
  - no TSH/T4/T3 data.

- 2-gen rat oral dietary study:
  - ↑ thyroid hypertrophy/hyperplasia, males + females (F0 + F1)
  - ↑ altered colloid in thyroid, males + females (F0 + F1)
  - no TSH/T4/T3 data.

In general, elevations in TSH occur with increased thyroid hypertrophy/hyperplasia. The T4 clearance hypothesis is also indirectly supported by liver enzyme induction data, altered gene expression targets indicative of CAR activation and a lack of effect on organification of iodine.

Overall, RAC agrees with the DS, the observed thyroid tumours are not considered to be of relevance to humans. RAC is of the opinion that the most plausible MoA is based on CAR/PXR-mediated induction of hepatic Phase II glucuronyltransferases leading to increased biliary clearance of T3/T4 glucuronides. As a consequence, blood levels of T3/T4 drop, triggering TSH-mediated stimulation of the thyroid follicular cells to increase production of T3/T4 via a negative feedback loop. TSH-mediated stimulation of thyroid follicular cells lead to an adaptive hyperplasic response, which if sufficiently prolonged can lead to tumour induction in these cells.

Relevance of tumour type for human hazard assessment

Background

Nuclear receptors such as the constitutive androstane receptor (CAR) and pregnane X receptor (PXR) are involved in the regulation of cellular responses from exposure to many xenobiotics (e.g. phenobarbital, carbamazepine, nicardipine, polycyclic and polyhalogenated aromatic hydrocarbons) and endogenous substances (steroid derivatives). In addition to inducing hepatic drug metabolism, acute CAR activation in rodents results in rapid, but transient and strictly limited liver growth. CAR activators, including phenobarbital (PB), are non-genotoxic carcinogens and liver tumour promoters in rodents, and CAR is required for their tumorigenic effects.

Data for two modes of action (MoA) have been presented, one concerning the promotion of rat liver tumours and the other concerning the promotion of rat thyroid follicular cell tumours. Both MoAs propose a central role for CAR/PXR activation that results in qualitative/quantitative differences in kinetic and dynamic factors between experimental animals and humans such that promotion of tumour development in these cases may be considered of little to no relevance for human hazard assessment (figure below).

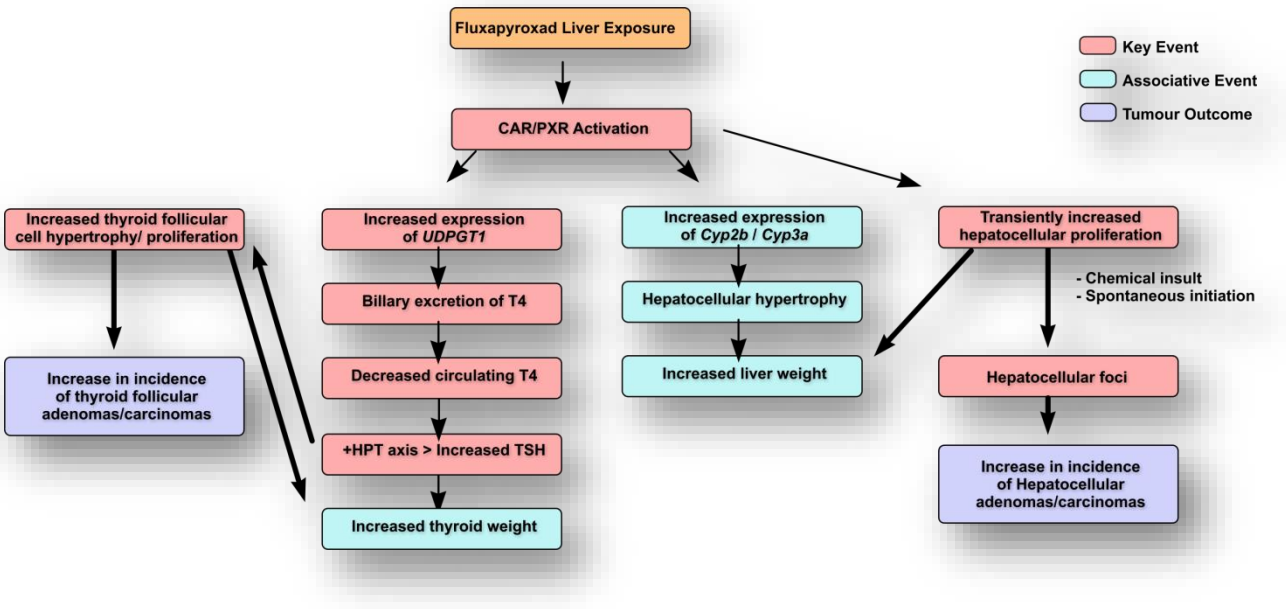


Figure: *Outline of the proposed mechanisms of action for fluxapyroxad-induced tumours in rat liver and thyroid*.

Liver tumours

The non-genotoxic MoA proposed for fluxapyroxad-induced rat liver tumours is CAR/PXR activation resulting in altered expression of CAR-responsive genes leading to CAR-mediated stimulation of cell proliferation (and associated replicative DNA synthesis). This promotes an environment permissive for increased cell replication, which can result in a higher rate of spontaneous mutations due to normal replication errors and increased altered hepatic foci. Combined with suppression of apoptosis (another feature of CAR activation), this promotes an environment that would allow a spontaneously mutated cell to clonally expand before it could be removed by normal apoptotic control processes. Over time, transformed cells progress to pre-neoplastic foci, with clonal expansion eventually leading to the development of liver tumours. The activation of CAR and subsequent burst of cellular proliferation are considered to be key events in the tumour MoA.

CAR activation also results in the induction of a number of other genes, including some coding for members of specific cytochrome P450 families of isozymes, particularly those of Cyp2b and, to a lesser extent, Cyp3a and Cyp2a subfamilies. The effects on cytochrome P450s are considered to be associative events in that while they are a characteristic hallmark of CAR activation, they are not central to the induction of liver tumours, i.e. they are not the cause of tumour promotion. A further associative event is liver hepatocellular hypertrophy, which is caused by proliferation of the smooth endoplasmic reticulum as a consequence of cytochrome P450 induction. This hypertrophy, in combination with the increased hepatocyte proliferation, in turn results in an increase in liver weight.

The MoA is considered not relevant for human hazard assessment purposes as regards tumour development, due to qualitative differences in toxicodynamics in response to CAR activation between rodents and humans (*Elcombe et al., 2014; Lake, 2018*). Experimental data demonstrate that fluxapyroxad does not produce the key event of cell proliferation induced by CAR activation in human liver cells *in vitro*. In contrast, *in vivo* rat dietary studies and *in vitro* rat hepatocyte studies show hepatocyte proliferation. Based on this species difference in response, fluxapyroxad is unlikely to cause cell proliferation in humans *in vivo*, and it is therefore unlikely to cause tumours in humans.

Thyroid tumours

The non-genotoxic MoA proposed for fluxapyroxad-induced rat thyroid tumours is CAR/PXR activation resulting in hepatic enzyme induction (UGT1A1 and others), leading to enhanced T4 metabolism/clearance. The resulting decrease in serum T4 stimulates the HPT axis to re-establish thyroid hormone homeostasis by increasing circulating levels of TSH, which induce thyroid follicular cells to produce and release more thyroid hormone. Increased TSH stimulation produces a variety of morphological and functional changes in the follicular cell including follicular cell hypertrophy, hyperplasia, and subsequently leads to thyroid follicular adenomas and carcinomas. In such cases thyroid tumours are therefore secondary to liver effects (hepatic uptake and clearance of thyroid hormones).

The development of thyroid tumours in this manner is considered to be not relevant to humans. Indeed, under CLP guidance one of the mechanisms of tumour formation considered not relevant for humans is thyroid tumours in rodents mediated by UDP glucuronyltransferase (UGT) induction (see section 3.6.2.3.2 part k; IARC, 1999; EU Specialised Experts, 1999). Many publications on this mechanism describe how human relevance can be reasonably excluded based on quantitative

differences in kinetic and dynamic factors between experimental animals and humans (e.g. *Bartsch et al., 2018*[1]). In many cases certain points are routinely noted such as:

1. In humans, to date, no chemical is known, which increases the incidence of thyroid tumours. Ionizing radiation is the only known human thyroid carcinogen. Phenobarbital has been used for about a century as a sedative, hypnotic and anti-epileptic substance. Yet compounds such as PB are model non-genotoxic carcinogens and tumour promoters in rodents.
2. Several pharmaceutical compounds (e.g. phenobarbital, phenytoin and carbamazepine) have been shown to induce - both in the rats and humans - hepatic enzyme activity resulting in reduced thyroid hormone levels (*Curran & DeGroot, 1991*). Yet despite the low thyroid hormone levels the TSH levels in humans remain mainly unaltered, whereas in the rat system the TSH levels invariably increase.
3. Thyroid hormone reserves are smaller in rats than humans, making the HPT axis in rats more sensitive to perturbations. Humans have a greater buffering capacity for thyroid hormone changes than rats (*Dellarco et al., 2006*).
4. Human TSH levels are more stable in response to exposure to hepatic enzyme activating agents (*Dellarco et al., 2006, Meek et al., 2003*).
5. Rats have a shorter thyroid hormone half-life due to the absence of thyroxine-binding globulin (T4 half-life is 5–9 days in humans vs. 0.5–1 day in rats, *Jahnke et al., 2004*); therefore, the rat HPT axis is more sensitive to feedback regulation and homeostatic mechanisms. For example, rats have higher (approx. 25-fold) baseline TSH levels than humans, reflecting the higher activity of the HPT axis in rats (*McClain, 1992; Finch et al., 2006*).
6. The increased rate of T4 clearance results in a more ''functionally active'' thyroid in rats than humans, which is reflected in different thyroid histology between the two species (*Dellarco et al, 2006*). Whereas in humans the thyroid follicular epithelium is composed of short cuboidal cells (indicative of their quiescent nature), the rat follicular cells are tall cuboidal and appear to be continuously active in synthesis. Rat follicular cells are considered more likely to undergo hyperplasia in response to TSH than humans due to the already stimulated state of the rat's follicular cells.
7. Humans have a very low incidence of thyroid tumours, whereas rats frequently develop thyroid tumours during chronic studies.

Conclusions

Classification into category 1A

There is no information from studies in humans to inform on carcinogenic potential and so classification in category 1A is not warranted.

Classification into category 1B

The substance was not found to be genotoxic. Tumours were restricted to one species, there was no reduction in liver tumour latency, liver tumours were seen in both sexes but thyroid tumours

[1] Bartsch et al., 2018. Human relevance of follicular thyroid tumors in rodents caused by non-genotoxic substances. Regulatory Toxicology and Pharmacology, Vol 98, 2018, Pages 199-208.

were confined to males. The incidence of thyroid adenomas was well within the historical control range. Overall the data was considered to show limited evidence of a carcinogenic effect and not sufficient to warrant classification in category 1B.

Classification into category 2

There is evidence of carcinogenicity in rats, but not in mice.

RAC considers the liver tumours to be of greater concern (positive dose-response, incidences above historical controls, both sexes affected, statistical significance) than the thyroid tumours (one sex affected, no statistically significant increase in either adenoma or carcinoma, incidence of adenoma within historical controls, incidence of carcinoma slightly above historical controls (1 extra case)). The thyroid tumours alone are considered not to constitute enough evidence for classification. Mechanistic data provide a further argument that no classification is warranted for the thyroid tumours, considering that the most plausible MoA is via CAR activation and UDPGT induction.

The data from the rat 2-year chronic toxicity/ carcinogenicity study regarding liver tumours on first inspection would suggest a category 2 classification is appropriate for fluxapyroxad. However, while not all key or associated events were proven (e.g. no altered foci observed in rats) or investigated, the data from several mechanistic studies point to a MoA via CAR(/PXR) activation as the most plausible mechanism behind the liver tumours (adenomas and carcinomas in males, adenomas in females). This mode of action of tumour formation is considered to be not relevant to humans. The evidence appears to support a downgrading of a Category 2 classification to no classification.

No Classification

Liver tumours: RAC considers the MoA for liver tumours in rats is secondary to hepatocellular proliferation induced by activation of the CAR/PXR nuclear receptors.

In this case, the liver tumours resulting from the CAR(/PXR)-mediated MoA are of little relevance to humans, given the difference observed in the prerequisite step for tumour formation, i.e. DNA replication: there is some limited evidence that DNA replication does not seem to occur with fluxapyroxad in human hepatocytes (from 1 donor only) following (weak) induction of human CAR/PXR, in contrast to rats. Accordingly, it appears the rat liver tumours would not pose a cancer hazard to humans, and therefore do not provide sufficient evidence for classification.

Thyroid tumours: RAC considers the MoA to be CAR/PXR induction of UDPGT isoforms with consequent elevation of TSH levels, thyroid follicular cell hyperplasia and eventual progression to follicular cell tumours. Under CLP guidance one of the mechanisms of tumour formation considered not relevant for humans is thyroid tumours in rodents mediated by UDP glucuronyltransferase (UGT) induction (see CLP guidance, v5 (2017) section 3.6.2.3.2).

In conclusion RAC proposes no classification of fluxapyroxad for carcinogenicity.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

The effects of fluxapyroxad were extensively described by the DS in table 37 of the CLH report. Fertility/sexual function were investigated in a two-generation reproductive toxicity study performed according to the guidelines (OECD TG 416) under GLP. fluxapyroxad (purity 99.7%)

was administered to Han Wistar rats in the diet giving rise to dose levels of 0, 10, 50, and 300 mg/kg bw/day (Anon. 2009i). This study was considered to be acceptable by the DS and RMS in the substance 2011 plant protection product DAR.

Table: *Summary description of Rat dietary 2-generation reproduction study. See table 37 of the CLH report for further detail.*

Study	Comments	Reference
Rat 2-gen; strain: Crl:WI(Han)	Oral (dietary) Dose: 0, 10, 50, 300 mg/kg bw/day 25 x female and 25 x male per dose per generation ***Acceptable.*** GLP - Yes Guidelines - Yes	Anon. (2009i).

General Effects

F0 Maternal toxicity

There were no treatment-related deaths. There were no clinical signs except for whitening of the maxillary or mandibular incisors. General toxicity was reported at the highest doses and included:

- ↓ body weight relative to controls (-6 to -10%) in all periods from pre-mating week 5 to post-natal day 21, 300 mg/kg bw/day, stat. sig.
- ↓ food consumption (at 50) and 300 mg/kg bw/day (-2 to -10.4%), stat. sig.
- ↑ GGT, marked at 300 mg/kg bw/day (males > females) → implies hepatobiliary system involvement (no histopathology data to substantiate the effect)
- ↑ absolute and relative thyroid weights (+9%, +25%**), 300 mg/kg bw/day (with ↑ hypertrophy/hyperplasia, follicular – diffuse).
- ↑ absolute and relative liver weights (+43%**, +57%**), 300 mg/kg bw/day (with ↑ hepatocellular centrilobular hypertrophy).
- ↑ absolute and relative liver weights (+10%**, +19%**), 50 mg/kg bw/day (with ↑ hepatocellular centrilobular hypertrophy).

F1 pup effects/toxicity

The effects found in F1 pups at the highest dose included:

- Lower body weight from PND 1 to PND 21:
  PND 1: ↓ BW 10%/10% (m/f)*
  PND 21: ↓ BW 19%/17% (m/f)*
  Body weight change PND 4-21: 20%/18% (m/f)*
- Lower absolute and/or relative brain, thymus and spleen weights secondary to lower pup weight
- Slight delay of preputial separation in males: 40.2, 40.0, 40.4, 41.4** days at 0, 10, 50, 300 mg/kg bw/day secondary to lower body weight (HCD 41.5 - 45.0 days)
- Average number of days to vaginal opening not affected.

F1 Maternal toxicity

The findings reported for the F1 dams were:

- ↓ body weight (-7 to -11% relative to controls) in all periods from pre-mating week 5 to post-natal day 21, top dose, stat. sig.
- ↓ food consumption at 300 mg/kg bw/day (-2.5 to -8%)

- ↑ GGT, marked at 300 mg/kg bw/day (males > females)
- ↑ absolute and relative thyroid weights (+13%, +25%**), 300 mg/kg bw/day (with ↑ hypertrophy/hyperplasia, follicular – diffuse)
- ↑ absolute and relative liver weights (+46%**, +63%**), 300 mg/kg bw/day (with ↑ hepatocellular centrilobular hypertrophy)
- ↑ absolute and relative liver weights (+9%**, +14%**), 50 mg/kg bw/day (with ↑ hepatocellular centrilobular hypertrophy)

F2-pups effects/toxicity

The effects found in F2 pups at the highest dose included:

- Lower body weight from PND 7 to PND 21:
  PND 21: ↓ BW 12%/12% (m/f)*
  Body weight change PND 4-21: 13%/13% (m/f)*
- Lower absolute and/or relative brain, thymus and spleen weights secondary to lower pup weight

Sexual function/fertility Effects

Fertility and mating indices were not affected by treatment (see additional key elements, table below). Fluxapyroxad had no effects on the oestrous cycle, the number, morphology and motility of sperm as well as on male or female reproductive performance, at doses of up to 300 mg/kg bw/day, the highest dose tested.

Duration of gestation was slightly reduced (though statistically significant), only in the F0 generation at 50 and 300 mg/kg bw/day (22.5/ 22.5/ 22.1**/ 22.1** days, at 0, 10, 50, 300 mg/kg respectively, table below); no historical control data was provided. This minor effect is not considered sufficient for classification.

There were no malformations and no effects on litter size, sex ratio or survival of pups. Viability and lactation indices were both in the range of 97 to 100%. Other pup parameters, including sex ratio, clinical observations, organ weights and gross necropsy findings did not reveal any treatment-related effects (see additional key elements, table below).

Conclusions

The DS did not propose classification for fertility.

Development

The developmental effects of fluxapyroxad were extensively described by the DS in table 38 of the CLH report.

Rat developmental toxicity

A developmental toxicity study was performed in rats in accordance with OECD TG 414. Female rats were mated and administered 0, 25, 200 or 1000 mg/kg bw/d fluxapyroxad by gavage (purity 99.2%) from day 6-19 of gestation. The females were killed on GD 20 of gestation and a necropsy was conducted.

There were no maternal deaths or clinical signs of toxicity. Maternal toxicity was very limited and expressed as a reduction in maternal bodyweight gain and food consumption at 200 and 1000 mg/kg/day and confined to effects seen from GD 6 – 8. Within this short time interval significant reductions in both parameters were observed and are outlined in the bullet points below:

- ↓ body weight gain [GD 6 – 8]
  1000 mg/kg bw/d: reduced by 35%** relative to control
  200 mg/kg bw/d: reduced by 25%* relative to control
- ↓ food consumption [GD 6 – 8]
  1000 mg/kg bw/d: reduced by 17%** relative to control
  200 mg/kg bw/d: reduced by 11%* relative to control

However, total bodyweight gain over the entire dosing period of gestation (GD 6 – 20), corrected for gravid uterus weight was not significantly different from controls at any dose. Treatment-related increases in liver and thyroid weights were observed as seen in all rat studies for fluxapyroxad. There were no toxicologically significant changes noted in clinical chemistry or general toxicity in the dams.

The pregnancy rate was slightly reduced at 1000 mg/kg bw/day; 25, 22, 23 and 21 dams were pregnant at 0, 25, 200 and 1000 mg/kg bw/day, respectively. None of the pregnant dams aborted or gave premature birth. Numbers of corpora lutea, implantation sites, pre- and post-implantation losses, litter size, sex ratios and foetal weights were comparable between controls and treated groups and within the historical control ranges. No treatment-related skeletal or visceral malformations or external malformations, or retardations of development were observed at doses of up to 1000 mg/kg bw/day, the highest dose tested (see additional key elements, table below).

Rabbit developmental toxicity

A developmental toxicity study was performed in rabbits in accordance with OECD TG 414. Female Himalayan rabbits were artificially inseminated and administered 0, 10, 25 or 60 mg/kg bw/d fluxapyroxad by gavage (purity 99.2%) from day 6-28 of gestation. The females were killed on GD 29 of gestation and the uterine contents were examined.

There were no deaths considered to be directly caused by fluxapyroxad. One female at 25 mg/kg bw/day died prematurely, on GD 26; the cause of death was not known. One female at 60 mg/kg/day died on GD9 as a result of a gavage error. One female at 60 mg/kg bw/day aborted on GD 29, having been observed with reduced/no faecal output from GD 19. As abortion is known to occasionally occur spontaneously in Himalayan rabbit developmental toxicity studies, this single observation was not considered to be treatment related.

The high dose of 60 mg/kg bw/day elicited some signs of maternal toxicity. Maternal bodyweight at the highest concentration tested (60 mg/kg bw/day) was significantly lower than controls from GD 16 (approximately 6% less than control) and food consumption was significantly reduced (-31% to –44% of controls on GD 10-11, GD 14-15, GD 18-19, $p \leq 0.01$) from a few days into the dosing period to around GD 20 and corroborated by an increased number of animals with reduced or no defecation.

The pregnancy rate was unaffected; 23, 24, 25 and 23 females were pregnant at 0, 10, 25 and 60 mg/kg bw/day, respectively. There were no treatment-related differences in the number of corpora lutea, implantation sites, pre-implantation losses, litter size, sex ratios and foetal weights. However, post-implantation loss at 60 mg/kg bw/day (21.1%) was significantly greater than controls (5.6%). The DS noted that the group mean was exaggerated by the presence of two mothers (2/ 21) with single implants which were lost, resulting in a 100% resorption rate for each of these females. The DS further noted that the exclusion of these two dams from calculation may result in post-implantation loss / early resorption values within the historical control range (a quick calculation by RAC indicates that a mean post-implantation loss of approximately 13% would be expected for the high dose does if account is taken of the two does with single implants lost). The historical control data was not provided by the DS but it is generally highly variable for this developmental endpoint in rabbits. According to calculations performed with the data from

Matsuo & Kast, 1995[1], the mean post-implantation loss for Himalayan rabbits would be expected to lie somewhere between 12.4 and 12.7%.

There were no treatment-related visceral or skeletal malformations observed (see additional key elements, table below). The only treatment-related variation was a statistically significant increase in incidence of mean % affected foetuses with paw hyperflexion (10.3%, HCD: up to 6.7%) at the high dose level (60 mg/kg bw/day). This is considered a minor variation and temporary change since tendons stretch postnatally and may be regarded with doubtful toxicological significance.

Conclusions

The DS did not propose classification for adverse effects on development.

Comments received during public consultation

No comments were received on reproductive toxicity.

Assessment and comparison with the classification criteria

Assessment of reductions in postnatal pup body weight

The DS did not propose to classify for effects on or via lactation.

In the 2-generation rat study fluxapyroxad treatment had a significant adverse effect on postnatal pup bodyweight in the absence of maternal toxicity. Pup bodyweights of the F1 generation showed a dose-related reduction (stat. signif.) in comparison with controls after birth from PND1 and throughout lactation at 50 and 300 mg/kg bw/day. Postnatal F2 pup bodyweights were reduced (stat. signif.) from PND7 onwards at 300 mg/kg bw/day (table below).

***Table**: Rat F1 and F2 postnatal bodyweight data*

Parameter	Nominal dose level (mg/kg bw/day)							
	F_1 generation offspring				F_2 generation offspring			
	0	**10**	**50**	**300**	**0**	**10**	**50**	**300**
Number of litters [n]	23	22	25	25	23	25	21	24
Live litter size [mean, n]	11.8	11.9	11.3	11.2	11.9	11.1	11.4	11.8
Male pup wt (g)								
- PND 1	7.0	6.7	**6.5***	**6.3***	6.5	6.8	6.6	6.1
- PND 7	17.6	16.9	**15.9****	**14.5****	16.1	16.6	16.0	**14.5****
- PND 14	33.9	33.7	**31.6****	**27.9****	32.3	32.6	32.9	**27.8****
- PND 21	54.7	54.4	**50.7****	**44.4****	50.3	51.5	51.7	**44.1****
Female pup wt (g)								
- PND 1	6.7	6.3	**6.2***	**6.0****	6.2	6.4	6.2	5.8
- PND 7	16.9	16.4	**15.5****	**14.3****	15.6	16.2	15.5	**14.0****
- PND 14	32.9	32.8	**30.8****	**27.4****	31.4	32.1	32.0	**27.1****
- PND 21	52.7	52.4	**48.8****	**43.5****	48.5	50.5	49.8	**43.4****

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

[1] *Matsuo & Kast,* (1995) Two decades of control Himalayan rabbit reproductive parameters and spontaneous abnormalities in Japan. Lab Anim. Jan; 29(1):78-82

The postnatal pup reduction in bodyweights relative to controls is consistent across two generations:

- F1 male pups PND 1-21, reduced bw, range → 10-19%
- F2 male pups PND 1-21, reduced bw, range → 6-14%
- F1 female pups PND 1-21, reduced bw, range → 10.5-17.5%
- F2 female pups PND 1-21, reduced bw, range → 6.5-13.7%

This effect may be considered adverse, and it is therefore important to consider whether it qualifies for classification for developmental toxicity or for effects on or via lactation. There are several important observations to note when assessing this effect:

1. The F0/F1 parental generation also shows a reduced body weight relative to controls with fluxapyroxad treatment, the magnitude of the effect is a reduction of about 7-10%. The effect is common to pups regarding postnatal growth.
2. There was no *in-utero* effect on mean foetal body weight. There was no effect on prenatal body weight at doses up to 1000 mg/kg bw/day in the rat developmental study.
3. There was no loss in body weight amongst pups from any dose group. All pups continued to thrive throughout PND 1-21.
4. As the pups matured the differences in body weight relative to controls generally diminished to non-significant levels except for top dose F_1 females (table below).
5. The onset of puberty was not significantly affected from a biological point of view (table below). There was a small but significant delay in preputial separation by about 1 day at 300 mg/kg bw/day that may be considered secondary to the lower bodyweight. This was within the historical control range (41.5 to 45.0 days). No treatment-related effects on vaginal opening were seen in the selected female F1 pups.
6. There were no adverse effects on fertility in the F1 males and females selected to breed the F2 generation.
7. The survival of pups was not affected as viability and lactation indices were both in the range of 97 to 100%.
8. Litter size and sex ratios for both the F1 and F2 generation offspring were similar to controls (table above).

***Table**: F1 generation: preputial separation and vaginal opening*

Parameter	Nominal dose level (mg/kg/day)							
	0	**10**	**50**	**300**	**0**	**10**	**50**	**300**
	F1 males: preputial separation				**F1 females: vaginal opening**			
Number of animals	25	25	25	25	25	25	25	25
Days to criterion	40.2	40.0	40.4	**41.4****	30.4	31.1	30.4	31.0
Body weight at criterion [g]	163.4	164.9	164.0	159.8	92.7	94.5	90.3	**86.4***

While the postnatal body weight data suggests that fluxapyroxad had indeed an adverse effect, it may be seen from the rat 2-generation and developmental studies that this effect was without significant toxicological consequence as the pups matured into adulthood. The reduced body

weight impacted in a minor way on preputial separation in males (slightly delayed puberty but within HCD), but otherwise it may be considered to have no impact on maturation or on parental fertility.

According to the CLP criteria, clear evidence in the offspring due to transfer to the milk or adverse effect on the quality of the milk or indications that the substance is present at potentially toxic levels in breast milk justifies classification for effects on or via lactation.

The contribution of the nursing behaviour was unclear, though clinical assessments of the dams suggest no ill health and therefore no reason to advocate that delivery of milk was a problem. Fluxapyroxad is a moderately lipophilic substance, as evidenced by its physical and chemical properties, and as such cannot be excluded that might be transferred to milk. But there were no studies to substantiate this. The reduction in body weight gain leading to a reduction in body weight relative to control pups at doses ≥ 50 mg/kg bw/day showed a dose response in F1 pups and occurred after birth. Reductions were also seen in F2 pups at 300 mg/kg bw/day from PND-7. The effects on pups occurred during the time period when the mother provided the sole means of nutrition. RAC considers the effect on postnatal body weight to be treatment-related. For RAC, the reduced postnatal bodyweight gain in two generations where milk is the only nutrition source justifies classification for effects on or via lactation.

Consideration of Category 1A classification

According to the CLP criteria, classification in Category 1A is largely based on evidence from human data, which were not present in the CLH report. Therefore, classification as Repr. 1A is not warranted.

Consideration of Category 1B classification

Categories 1B and 2 are reserved for presumed and suspected human reproductive toxicants, respectively, and must be based on the presence of clear (Category 1B) or some (Category 2) evidence of alterations in sexual function, fertility, or development.

There is no clear evidence of alterations in sexual function, fertility, or development amongst the rat and rabbit studies submitted as part of the fluxapyroxad toxicology dossier. Classification as Repr. 1B is not warranted.

Consideration of Category 2 classification

<u>Sexual function and fertility</u>

There were some general toxicity findings in the two-generation rat study mainly relating to body weight parameters but no toxicologically significant effects on reproductive performance or fertility were observed. Treatment with fluxapyroxad had no effects on the oestrous cycle, the number, morphology and motility of sperm as well as on male or female fertility or reproductive performance. The survival of pups was not affected by treatment as viability and lactation indices were both in the range of 97 to 100%. Other pup parameters, including sex ratio, clinical observations, organ weights and gross necropsy findings did not reveal any treatment-related effects.

The slight delay in preputial separation in F1 pups and the slight reduction in gestational length of F0 dams were not considered as sufficiently adverse for classification.

In general, fluxapyroxad had no effect on the reproductive system and classification for fertility is not warranted.

Development

In the rat there were no treatment-related skeletal or visceral malformations or external malformations, or retardations of development observed in pups from dams at doses of up to 1000 mg/kg bw/day.

In the rabbit, no treatment-related visceral or skeletal malformations were observed. The only treatment-related variation was a statistically significant increase in incidence of mean % affected foetuses with paw hyperflexion (10.3%) at the high dose level (60 mg/kg bw/day). This was considered a minor and transient effect.

There is some concern for the increased post-implantation losses in the rabbit study, which are supported by a dose-response (even if discounting the 2 dams with total loss). However, the effect may be regarded as slight and not sufficiently robust for classification.

Information from reliable animal studies in two species showed that fluxapyroxad had no effects of sufficient and significant concern on developmental properties. Therefore, the criteria for classification are not met and no classification for developmental toxicity is proposed.

Lactation

The significant adverse effect on rat F1 and F2 postnatal pup bodyweight seen in the 2-generation study may be viewed in the context of an important postnatal growth delay but without significant impact on later maturation and fertility. The effect was considered as sufficiently adverse to **classify fluxapyroxad for effects on or via lactation, Lact.; H362**.

Conclusion

RAC concludes that **no classification is warranted for adverse effects on sexual function and fertility or on development**. RAC concludes to **classify for effects on or via lactation, Lact.; H362.**

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Fluxapyroxad is a pesticidal active substance and currently used as a fungicide on various crops. Fluxapyroxad does not have an existing entry in Annex VI of CLP and at the time of CLH submission, has not been registered under REACH. The CLH dossier presents a classification and labelling proposal based on data submitted in the context of the application for approval as an active substance under the PPP regulation.

Based on available data the dossier submitter (DS) considered Fluxapyroxad as not rapidly degradable, to have a low potential for bioaccumulation and proposed an environmental hazard classification as Aquatic Acute 1 (H400) with an M-factor of 1 based on the lowest acute aquatic toxicity to the fish *Cyprinus carpio* (96-h LC50 = 0.290 mg/L), and Aquatic Chronic 1 (H410) with an M-factor of 1, based on chronic aquatic toxicity for *Pimephales promelas* (33 d FELS NOEC = 0.0359 mg/L). As *Pimephales promelas* were not the most sensitive fish species in acute testing and chronic fish data was not available for *Cyprinus carpio*, the DS noted that if new data will become available, the chronic M-factor may need to be reconsidered.

Degradation

In a ready biodegradability study following OECD TG 301 B, biodegradation of Fluxapyroxad was observed to be <10% of the theoretical CO_2 value after 28 days (Schwarz H., 2008). Therefore, the DS concluded that Fluxapyroxad should be considered as not readily biodegradable.

A preliminary hydrolysis test at 50°C over 5 days was conducted according to OECD TG 111 in sterile aqueous buffer solutions at environmentally relevant acidic, neutral and alkaline conditions (pH 4, 5, 7 and 9). Fluxapyroxad was found to be hydrolytically stable at all four pH values with less than 10% hydrolysis of the test item over the 5-day period for all pH values. Therefore, the main test at 25°C was not performed. As less than 10% hydrolysis was observed, the hydrolysis DT_{50} at 25 °C was considered greater than one year (Hassink, 2009a).

The degradation of Fluxapyroxad was investigated under dark and irradiated conditions in two different natural water/sediment systems (Berghäuser Altrhein and Ranschgraben) according to OECD TG 308 (Ebert D., 2009a).

The study demonstrated that Fluxapyroxad undergoes rapid partitioning from the water phase to sediment with limited further degradation. Overall, degradation was slow in water/sediment systems when incubated under dark conditions. The radioactivity in the water decreased from initially 87-94% AR (Applied Radioactivity) to 9-14% AR after 100 days. Correspondingly, the radioactivity in the sediment increased in both systems reaching 84-87% AR at the end of the incubation. After 100 days, Fluxapyroxad was found in the water at levels of 8-9% AR in the Berghäuser Altrhein system and 13-14% AR in the Ranschgraben system. Under dark conditions, the metabolite M700F002 was formed only in the Berghäuser Altrhein system in low amounts (4% TAR) towards the end of incubation. No other metabolites ever exceeded 2.1% AR including degradation products in sediment extracts. However, the degradation slightly increases in water/sediment systems when incubated under irradiation conditions. Metabolites M700F001 and M700F007 were formed in both systems (max. amounts of 11 and 7.5% AR, respectively). Despite the rapid primary degradation, minimal mineralization was observed. A maximum of 1.1% AR as CO_2 was observed in the dark experiment by day 100 with a maximum of 2.8% AR as CO_2 in the irradiated experiment by termination on day 57.

The DT_{50} (converted to 12 °C) values in the water phase were ≤ 13 days reflecting dissipation to the sediment phase. In the sediment, DT_{50} (converted to 12 °C) values could only be obtained for the irradiated experiment.

	Dark experiment (12 °C)	Irradiated experiment (12 °C)
DT_{50} (whole system)	1316 to >1896 days	355 to 444 days
DT_{50} (water)	6.4 to 9.7 days (dissipation)	6.4 to 13.3 days (dissipation)
DT_{50} (sediment)	could not be calculated	225 to 328 days

Aqueous photolysis studies indicate that Fluxapyroxad is stable in water at pH 7 with and without influence of light (Hassink, 2009b) and the metabolite M700F007 is stable in sterile natural pond water at pH8 with and without influence of light (Hassink, 2009c).

Overall, the degradation information from the study does not provide sufficient data to show that Fluxapyroxad is ultimately degraded (mineralised) within 28 days (equivalent to a half-life < 16 days) or undergoes primary degradation to non-classifiable products with half-lives < 16 days. Consequently, DS considered Fluxapyroxad as not rapidly degradable for the purpose of classification and labelling.

Aquatic Bioaccumulation

The measured log P_{ow} for Fluxapyroxad (99.3% purity) was 3.13 at pH 7 and 20 °C (Wilfinger, 2008). This value is below the CLP trigger of ≥ 4 and indicates a low potential for bioaccumulation. Available BCF values were less than the CLP criterion of 500 for both Fluxapyroxad and total radioactive residue (TRR). Whole fish BCF steady state lipid normalised to 5% lipid were 46 to 47 L/kg based on parent and 110 to 119 L/kg based on TRR. Therefore DS proposed not to consider Fluxapyroxad as bioaccumulative.

Aquatic Toxicity

The aquatic toxicity test results from available acute and chronic studies for all trophic levels of Fluxapyroxad are summarised in the following table and sections. Only the valid acute and chronic studies on Fluxapyroxad which are relevant for hazard classification purposes are included in the following table and relevant endpoints from these studies are discussed in further detail below.

Test organism / guideline, test method	Short-term result (endpoint)	Long-term result (endpoint)	Reference
Fish			
Rainbow trout (*Oncorhynchus mykiss*) OECD TG 203, GLP	96h LC_{50} = 0.546 mg/L (n verified)		Anonymous (2007)
Bluegill sunfish (*Lepomis macrochirus*) OECD TG 203, GLP	96h LC_{50} = 1.15 mg/L (mm)		Anonymous (2008i)
Fathead minnow embryos (*Pimephales promelas*) OECD TG 203, GLP	96h LC_{50} = 0.466 mg/L (mm)		Anonymous (2009x)
Common carp *(Cyprinus carpio)* MAFF No 12, Nosan No 8147, GLP	**96h LC_{50} = 0.290 mg/L (mm)**		Anonymous (2008j)
Sheepshead minnow (*Cyprinodon variegatus*) EPA 850.1075, GLP	96h LC_{50} = 1.30 mg/L (mm)		Anonymous (2009y)
Fathead minnow embryos (*Pimephales promelas*) OECD TG 210, GLP		**33d NOEC = 0.0359 mg/L (mm**)	Anonymous (2009z)
Aquatic invertebrates			
Water flea (*Daphnia magna*) OECD TG 202, GLP	48h EC_{50} = 6.78 mg/L (mm)		Janson, 2009a
Mysid shrimp (*Americamysis bahia*) EPA 850.1035; ASTM E 729, GLP	48h EC_{50} = 6.1 mg/L 96h EC_{50} = 3.6 mg/L (mm)		Gallagher *et al*., 2009a
Eastern oyster (Crassostrea virginica) ASTM E729, EPA 850.1025	96h $EC_{50\ shell\ deposition}$ = 1.1 mg/L 96h LC_{50} = >2.8 mg/L (mm)		Gallagher *et al*., 2009b
Water flea (*Daphnia magna*) OECD 211, GLP		21d NOEC = 0.500 mg/L (n verified)	Janson, 2009b

Test organism / guideline, test method	Short-term result (endpoint)	Long-term result (endpoint)	Reference
Mysid shrimp (*Americamysis bahia*) EPA 850.1350; ASTM E1191-97, GLP		29h NOEC = 0.30 mg/L (n verified)	Minderhout *et al*., 2010
Algae			
Algae *(Pseudokirchneriella subcapitata)* OECD 201, GLP	72h E_rC_{50} = 0.700 mg/L (n verified)	72h E_rC_{10} = 0.31 mg/L (n verified)	Hoffmann, 2008a, 2009a, 2010
Algae *(Anabaena flos-aquae)* OECD TG 201, GLP	72h E_rC_{50} = 2.61 mg/L (mm)	72h E_rC_{10} = 1.20 mg/L (mm)	Hoffmann, 2009b
Algae *(Navicula pelliculosa)* OECD TG 201, GLP	72h E_rC_{50} >3.42 mg/L (mm)	72h E_rC_{10} = 0.97 mg/L (mm)	Hoffmann, 2009c
Algae *(Skeletonema costatum)* OECD TG 201, GLP	72h E_rC_{50} =5.88 mg/L (mm)	72h E_rC_{10} = 0.69 mg/L (mm)	Hoffmann, 2009d
Toxicity to aquatic plants			
Lemna gibba / Draft OECD guideline, OECD TG 221, GLP	7d $E_rC_{50\ (frond\ number)}$ > 3.43 mg/L 7d $E_rC_{50\ (dry\ weight)}$ > 3.43 mg/L (mm)	7d $E_rC_{10\ (frond\ number)}$ 1.22 mg/L 7d $E_rC_{10\ (dry\ weight)}$ > 0.69 mg/L (mm)	Hoffmann, 2009f

mm = mean measured concentrations
n = nominal concentrations
verified refers to analytical concentrations within 20% of nominal values

The most acutely sensitive trophic group were fish with a lowest 96 hour LC_{50} value for *Cyprinus carpio* of 0.290 mg/L. *Oncorhynchus mykiss* and *Pimephales promelas* 96 hour LC_{50} values were slightly different (respectively 0.546 and 0.466 mg/l). These are complimented by a 72h E_rC_{50} value for algae Pseudokirchneriella *subcapitata* of 0.70 mg/L. However, all of these results are below the 1 mg/L cut-off to derive aquatic acute toxicity and in the same range for M-factor determination.

The most sensitive organism for chronic toxicity is also fish. In an early life stage (FELS) study with fathead minnow (*Pimephales promelas*) following OECD TG 210, the overall NOEC (33 d.) for Fluxapyroxad was determined to be 0.0359 mg/l based on mean measured concentrations. Nevertheless, the DS noted that *Pimephales promelas* were not the most sensitive fish species in acute testing and that chronic fish data are not available for *Cyprinus carpio*. As new data become available, the chronic M-factor may need to be reconsidered.

Overall, the DS proposed to classify Fluxapyroxad as:

Aquatic Acute 1 (H400), based on 96 hour LC_{50} value for *Cyprinus carpio* of 0.290 mg/L. As this value is in the range of 0.1 mg/L $< L(E)C_{50} \leq$ 1 mg/L, the M-factor should be 1.

Aquatic Chronic 1 (H410), based on 33-d NOEC value for *Pimephales promelas* of 0,0359 mg/l, as the substance is considered not rapidly degradable. As this value is in the range of 0.01 mg/L $< L(E)C50 \leq$ 0.1 mg/L, the M-factor should be 1.

Comments received during public consultation

Three MSCAs submitted comments on the environmental part of the DS's proposal. All of them fully agreed with the DS proposal without further justification.

Assessment and comparison with the classification criteria

Degradation

Biodegradation of Fluxapyroxad in the carbon dioxide evolution test was <10 % CO_2/$ThCO_2$ after an exposure period of 28 days (OECD TG 301B), so the substance is considered not readily biodegradable.

No hydrolysis of Fluxapyroxad was observed and substance was stable in water under environmentally relevant acidic, neutral and alkaline conditions (pH 4, 5, 7, 9) with <10% hydrolysis observed and the DT_{50} at 25 °C is considered to be greater than 1 year.

No photolysis of Fluxapyroxad was observed and the substance was stable in water at pH 7 and 22 °C with and without influence of light, as was the degradant M700F007 in sterile natural pond water at pH 8 and 22 °C.

In a water/sediment simulation study under dark conditions, the degradation of Fluxapyroxad was slow in two different natural water/sediment systems. The radioactivity in the water decreased from 87-94% AR to 9-14% AR after 100 days. Correspondingly, the radioactivity in the sediment increased in both systems reaching 84-87% AR at the end of the incubation. After 100 days, Fluxapyroxad was found in the water at levels of 8-9 and 13-14 % AR in two different systems. As well under dark conditions, the metabolite M700F002 was formed only at low amounts (4 % AR). Under dark conditions, the DT_{50} (converted to 12 °C) for the whole system was 1316 to >1896 days.

In a water/sediment simulation study under irradiation conditions, the degradation slightly increases. The metabolites M700F001 and M700F007 were formed in both systems (max. amounts of 11 and 7.5% AR). Under irradiation conditions, DT_{50} (converted to 12 °C) for whole system was 355 to 444 days.

Minimal mineralization was observed. A maximum of 1.1% AR as CO_2 was observed in in the dark experiment by day 100 with a maximum of 2.8% AR as CO_2 in the irradiated experiment by termination on day 57.

Overall, Fluxapyroxad undergoes rapid partitioning from the water phase to sediment in both systems. However given the high levels of AR and low levels of metabolites at study termination, further degradation of Fluxapyroxad is limited. The degradation information does not provide sufficient data to show that Fluxapyroxad is ultimately degraded to a level > 70 % within 28 days (equivalent to a half-life < 16 days) or transformed to non-classifiable products.

Consequently, RAC agrees that Fluxapyroxad should be considered as not rapidly degradable for the purpose of classification under the CLP regulation.

Aquatic Bioaccumulation

The measured log P_{ow} for Fluxapyroxad (99.3% purity) is 3.13 at pH 7 and 20°C which is below the CLP trigger of ≥ 4. The results of a bioconcentration factor (BCF) study with Bluegill sunfish were less than the CLP criterion of 500 for both Fluxapyroxad and total radioactive residue (TRR). Whole fish BCF steady state lipid normalised to 5% lipid were 46 to 47 L/kg based on parent and 110 to 119 L/kg based on TRR.

Therefore, RAC agrees with the DS's conclusion that the substance is not bioaccumulative.

Aquatic Toxicity

RAC notes that there are reliable acute and chronic aquatic toxicity data for all trophic levels. The most acutely sensitive trophic group were fish and one algae species had results in the same range. These acute toxicity results were slightly different but still in the same range for classification purposes and M-factor derivation. The most sensitive species for chronic toxicity were fish. However, RAC notes that the most sensitive species used under chronic toxicity testing were not the most sensitive fish species used under acute testing (*Cyprinus carpio*) and that chronic fish data are not available for *C. carpio*. Therefore, if available chronic data for the most acutely sensitive species will become available in the future, the chronic M-factor may need to be revised.

Overall, RAC agrees that the lowest acute endpoint for aquatic acute classification purpose is a 96 hour LC_{50} value for *Cyprinus carpio* of 0.29 mg/L based on mean measured concentrations. RAC further agrees that the lowest chronic endpoint for aquatic chronic classification is a 33-d NOEC for *Pimephales promelas* of 0.0359 mg/L, based on mean measured concentrations.

Conclusion on classification

Fluxapyroxad is considered not rapidly degradable and does not fulfil the criteria for bioaccumulation. Based on the available and most reliable information, **RAC agrees with the DS** that Fluxapyroxad should be classified as:

Aquatic Acute 1 based on LC_{50} = 0.290 mg/L for *Cyprinus carpio*. As this acute toxicity value falls within the $0.1 < L(E)C_{50} \leq 1$ mg/L range, the **acute M-factor is 1**.

Aquatic Chronic 1 based on NOEC =0.0359 mg/L for *Pimephales promelas*. As this chronic toxicity value falls within the $0.01 < NOEC \leq 0.1$ mg/L range, the **chronic M-factor is 1.**

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

The DS noted that Fluxapyroxad is a solid, with a corresponding extremely low vapour pressure. No boiling point could be determined before decomposition of the substance occurred. Hence, it is unlikely that Fluxapyroxad would be available in the stratosphere. It should be noted that Fluxapyroxad does not contain any halogen functionality other than fluorine. No specific data have been provided for hazard to the ozone layer, considering the chemical structure and other available information on the physico-chemical properties. Therefore DS conclude that Fluxapyroxad is not expected to be hazardous to stratospheric ozone.

Comments received during public consultation

No comments have been provided regarding to the hazards to the ozone layer.

Assessment and comparison with the classification criteria

A substance shall be classified as hazardous to the ozone layer (Category 1) if the available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer. RAC assumes that it is unlikely that Fluxapyroxad would be available in the stratosphere based on chemical structure and other available information on physico-chemical properties and consider that Fluxapyroxad does not contain any halogen functionality

other than fluorine and that there is no indication (data) that Fluxapyroxad may present a danger to the structure and/or the functioning of the stratospheric ozone layer.

Consequently, RAC agrees that Fluxapyroxad is **not expected to be hazardous to stratospheric ozone and does not require classification according to the CLP regulation**.

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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).