Committee for Risk Assessment
RAC

Annex 1

Background document

to the 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

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

Adopted

30 November 2018

CLH Report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

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

EC Number: **Not assigned**

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

Index Number: **Not assigned**

Contact details for dossier submitter: **UK Competent Authority**
Chemicals Regulation Division
Health and Safety Executive
United Kingdom

Version number: 2 **Date: December 2017**

Note on confidential information

Please be aware that this report is intended to be made publicly available. Therefore it should not contain any confidential information. Such information should be provided in a separate confidential Annex to this report, clearly marked as such.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 3-(DIFLUOROMETHYL)-1-METHYL-N-(3',4',5'-TRIFLUOROBIPHENYL-2-YL)PYRAZOLE-4-CARBOXAMIDE; FLUXAPYROXAD

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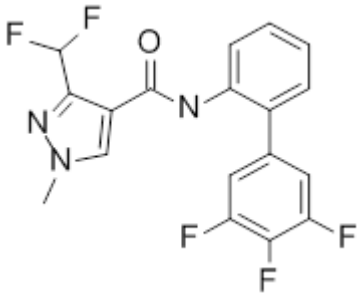
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	3-(difluoromethyl)-1-methyl-N- (3′,4′,5′-trifluoro [1,1′-biphenyl]-2-yl)-1H-pyrazole-4-carboxamide
Other names (usual name, trade name, abbreviation)	Fluxapyroxad, Xemium, BAS 700 F
ISO common name (if available and appropriate)	Fluxapyroxad (pending ISO publication)
EC number (if available and appropriate)	Not assigned
EC name (if available and appropriate)	Not assigned
CAS number (if available)	907204-31-3
Other identity code (if available)	CIPAC number: 828
Molecular formula	$C_{18}H_{12}F_5N_3O$
Structural formula	
SMILES notation (if available)	Cn1cc(C(=O)Nc2ccccc2c3cc(F)c(F)c(F)c3)c(n1)C(F)F
Molecular weight or molecular weight range	381.30 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 98%

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Fluxapyroxad; 3-(difluoromethyl)-1-methyl-N- (3′,4′,5′-trifluoro [1,1′-biphenyl]-2-yl)-1H-pyrazole-4-carboxamide CAS: 907204-31-3	≥ 98%	Not listed	Carc. 2* Aquatic Acute 1 Aquatic Chronic 1 Labelling: GHS09; GHS08 Signal word: Warning H351, H400, H410

**In accordance with the information provided in the EFSA conclusion*
The self-classification is in line with that presented in the C&L Inventory

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Toluene Index number: 601-021-00-3; EC Number: 203-625-9; CAS Number: 108-88-3 Max. 1 g/kg (based on pilot plant production) The relevant impurity should not exceed 0.6 g/kg in the technical material based on commercial plant production information.	< 0.1%	Classification: Flam. Liq. 2; Asp. Tox. 1; Skin Irrit. 2; STOT SE 3; Repr. 2; STOT RE 2 Labelling: GHS02, GHS08, GHS07 Signal word: Danger H225, H361d, H304, H373, H315, H336	Classification: Flam. Liq. 2; Asp. Tox. 1; Skin Irrit. 2; STOT SE 3; Repr. 2; STOT RE 2 Labelling: GHS02, GHS08, GHS07 Signal word: Danger H225, H361d, H304, H373, H315, H336	No. Due to the concentration at which it is present and the available data on fluxapyroxad it is not considered to contribute to the classification and labelling.

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

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
None	-	-	-	-	-

The batches used in the relevant studies were considered equivalent to the substance as described above.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	Not currently listed										
Dossier submitters proposal	TBD	3-(difluoromethyl)-1-methyl-N-(3',4',5'-trifluorobiphenyl-2-yl)pyrazole-4-carboxamide; fluxapyroxad	Not assigned	907204-31-3	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Warning	H410	None	M (acute) = 1 M (chronic) = 1	-
Resulting Annex VI entry if agreed by RAC and COM	TBD	3-(difluoromethyl)-1-methyl-N-(3',4',5'-trifluorobiphenyl-2-yl)pyrazole-4-carboxamide; fluxapyroxad	Not assigned	907204-31-3	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Warning	H410		M (acute) = 1 M (chronic) = 1	-

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Hazard not applicable substance is a solid	No
Oxidising gases	Hazard not applicable substance is a solid	No
Gases under pressure	Hazard not applicable substance is a solid	No
Flammable liquids	Hazard not applicable substance is a solid	No
Flammable solids	Data conclusive but not sufficient for classification	Yes
Self-reactive substances	Data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	Hazard not applicable substance is a solid	No
Pyrophoric solids	Data conclusive but not sufficient for classification	Yes
Self-heating substances	Data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	Yes
Oxidising liquids	Hazard not applicable substance is a solid	No
Oxidising solids	Data conclusive but not sufficient for classification	Yes
Organic peroxides	Hazard not applicable substance does not contain peroxide moities	No
Corrosive to metals	Data conclusive but not sufficient for classification	Yes
Acute toxicity via oral route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Data conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	Data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	Data Lacking	No
Skin sensitisation	Data conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	Data conclusive but not sufficient for classification	Yes
Reproductive toxicity	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification	Yes
Aspiration hazard	Hazard not applicable substance is a solid	No
Hazardous to the aquatic environment	Harmonised classification proposed	Yes
Hazardous to the ozone layer	Data conclusive but not sufficient for classification	Yes

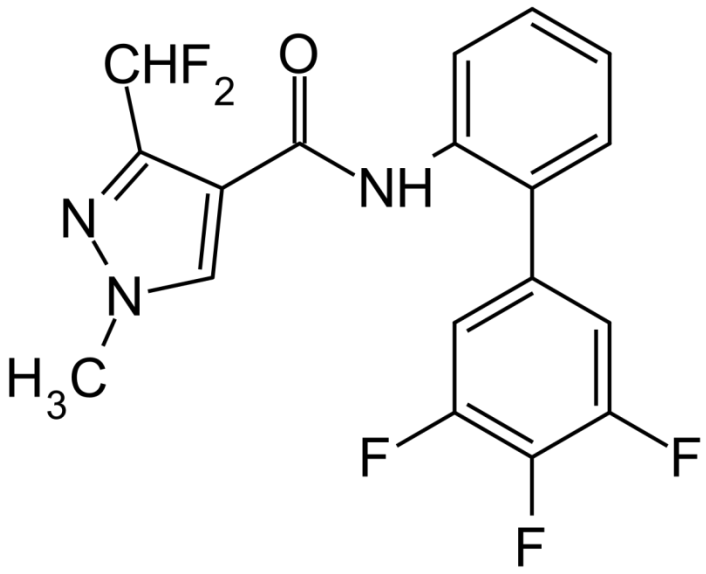
3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Fluxapyroxad is an active substance in the scope of Regulation 1107/2009. It has not been considered for harmonised classification and labelling in the EU previously.

In the EFSA conclusion (EFSA Journal 2012;10(1):2522) on the peer review of the pesticide risk assessment for fluxapyroxad, concern for classification as Carc 2; H351 was raised due to the observation of liver and thyroid tumours. Classification with Aquatic Acute 1: H400 and Aquatic Chronic 1; H410 was also noted. The substance is classified and labelled as such in the C&L Inventory.

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.



4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Fluxapyroxad is a pesticidal active substance in the scope of Regulation 1107/2009. As such, it is subject to the harmonised classification and labelling process in accordance with Article 36 (2) of CLP.

5 IDENTIFIED USES

Fluxapyroxad is currently used as a fungicide within and outside the EU. Fluxapyroxad is used in various crops in more than 55 countries worldwide.

6 DATA SOURCES

This evaluation relies on data submitted in the context of the application for approval as an active substance under Regulation 1107/2009. Additional information has been provided to address the proposed mode of action for the liver and thyroid tumours observed in the long-term rat study.

Information is also available in the EFSA conclusion on the peer review of the pesticide risk assessment of the active substance; EFSA Journal 2012;10(1):2522.

Full references are provided in section 14.

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

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	White solid (fine crystalline powder	2006/1036276 Kroehl T. 2006	Visual inspection (99.3 % pure)
Melting point	156.8 °C	2006/1036276 Kroehl T. 2006	OECD 102 (99.3 % pure)
Boiling point	No boiling point; decomposition at approx. 230°C (exothermic)	2006/1036276 Kroehl T. 2006	OECD 102 (DSC method) (99.3 % pure)
Relative Density	D^{20}_4 = 1.42	2006/1036276 Kroehl T. 2006	OECD 109; EC A.3.1.4.3 (99.3 % pure)
Vapour pressure	2.7 x 10^{-9} Pa at 20°C 8.1 x 10^{-9} Pa at 25°C	2006/1036276 Kroehl T. 2006	OECD 104; EC A.4 (99.3 % pure)
Surface tension	73.3 mN/m at 20°C, 1.3 mg/L (90% of the saturation solubility in pure water)	2008/1014896 Kroehl T. 2008	OECD 115; EC A.5 1.6.1 (99.4 % pure)
Water solubility	3.78 mg /L at 20°C (pH 4.01) 3.88 mg /L at 20°C (pH 5.84) 3.44 mg /L at 20°C (pH 7.00) 3.84 mg /L at 20°C (pH 9.00)	2007/1056999 Wilfinger W. 2008	OECD 105; EC A.6 (99.3 % pure)
Partition coefficient n-octanol/water	Log P_{ow} at 20°C: 3.08 (deionized water) 3.09 at pH 4 3.13 at pH 7 3.09 at pH 9	2007/1057001 Wilfinger W. 2008	OECD 117; EC A.8 (99.3 % pure)
Flash point	Not applicable, the test substance is a solid.		
Flammability	Not highly flammable. Brief burning and rapid extinction observed. No gas was observed in contact with air at 25°C for 5 mins. No gas evolved on contact with water.	2008/1070100 Loehr S. 2008	EC A.10, A.12, A13 Measurement and expert justification (99.4 % pure)
Explosive properties	Not explosive. The test substance did not exhibit any thermal or mechanical (shock and friction) sensitivity under the conditions of the test.	2008/1070100 Loehr S. 2008	EC A.14 Measurement and expert justification (99.4 % pure)
Self-ignition temperature	No self-ignition detected at < 400°C	2008/1070100 Loehr S. 2008	EC A.16 (99.4 % pure)

Property	Value	Reference	Comment (e.g. measured or estimated)
Oxidising properties	Not oxidizing. The maximum burning rate (2.35 mm/s) was lower than that of the reference material.	2008/1070100 Loehr S. 2008	EC A.17 Measurement and expert justification (99.4 % pure)
Granulometry	No data.		
Stability in organic solvents and identity of relevant degradation products	Solubility [g/L] at 20°C: acetone >250 acetonitrile 167.6 ±0.2 dichloromethane 146.1 ±0.3 ethylacetate 123.3 ±0.2 methanol 53.4 ±0.0 toluene 20.0 ± 0 .0 n-octanol 4.69 ±0.01 n-heptane 0.106 ±0.001	2007/1057003 Wilfinger W. 2008	OECD 105; EC A.6 Measurement
Dissociation constant	$pK_{a\ (HL/H+L)}$ = 12.58 ± 0.70 (calculated) Assessed with the aid of a validated ACD/Labs modelling software from Advanced Chemistry Development Inc.	2007/1057000 Wilfinger W. 2008	OECD 112 (calculation) Titration method is not suitable due to low water solubility. Spectrometric method is not suitable as no repeatable UV spectra could be determined. Therefore a calculation is used. (99.3 % pure)
Viscosity	Not applicable substance is a solid.		

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 8: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EC A.14	Thermal sensitivity: no explosion Mechanical sensitivity: no explosion Friction test: no explosion, no crepitation, no flames		2008/1070100 Loehr S. 2008

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

The test substance did not exhibit any thermal or mechanical (shock and friction) sensitivity under the conditions of the test.

8.1.2 Comparison with the CLP criteria

A substance is considered for classification as an explosive substance where a positive result is obtained in the test series indicated in figure 2.1.2 of Annex I of the CLP regulation. Fluxapyroxad was not found to be sensitive to the effects of heat, shock or friction. Consequently, it does not meet the criteria for classification as an explosive substance.

8.1.3 Conclusion on classification and labelling for explosive properties

Not classified – conclusive but not sufficient for classification.

8.2 Flammable gases (including chemically unstable gases)

Not relevant as the active substance is a solid.

8.3 Oxidising gases

Not relevant as the active substance is a solid.

8.4 Gases under pressure

Not relevant as the active substance is a solid.

8.5 Flammable liquids

Not relevant as the active substance is a solid.

8.6 Flammable solids

Table 9: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EC A.10	Brief burning followed by rapid extinction. Not highly flammable.		2008/1070100 Loehr S. 2008

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

On attempt of ignition only brief burning with rapid extinction was observed.

8.6.2 Comparison with the CLP criteria

A substance (non-metal) is classified as a flammable solid when the burning time is < 45 seconds or the burning rate is > 2.2 mm/s. On attempted ignition, only brief burning with rapid extinction was observed. Therefore, the criteria for classification as a flammable solid are not met.

8.6.3 Conclusion on classification and labelling for flammable solids

Not classified – conclusive but not sufficient for classification.

8.7 Self-reactive substances

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

The self-accelerating decomposition temperature (SADT) is estimated to be > 75ºC based on DSC measurements with an onset temperature of 290°C (energy release 30 J/g) and 335°C (energy release 950 J/g) respectively.

8.7.2 Comparison with the CLP criteria

A substance is considered to be self-reactive where the SADT is less than or equal to 75°C when transported in a 50 kg package.

On the basis of the presented DSC measurements, with an onset temperature of 290°C (energy release 30 J/g) and 335°C (energy release 950 J/g) respectively, the SADT can be estimated to be >75°C.

Therefore, fluxapyroxad is not considered to be self-reactive.

8.7.3 Conclusion on classification and labelling for self-reactive substances

Not classified – conclusive but not sufficient for classification.

8.8 Pyrophoric liquids

Not relevant as the active substance is a solid.

8.9 Pyrophoric solids

Table 10: Summary table of studies on pyrophoric solids

Method	Results	Remarks	Reference
EC A.13	No gas was observed in contact with air at 25°C for 5 mins.		2008/1070100 Loehr S. 2008

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

No gas was observed when fluxapyroxad was in contact with air at 25°C for 5 minutes. Evidence is also provided from experience in handling and use where the substance is known to be stable in contact with air at room temperature.

8.9.2 Comparison with the CLP criteria

A substance is classified as a pyrophoric solid if it ignites within 5 minutes of coming into contact with air. As this was not the case with fluxapyroxad, the criteria for classification are not met.

8.9.3 Conclusion on classification and labelling for pyrophoric solids

Not classified – conclusive but not sufficient for classification.

8.10 Self-heating substances

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

No self-ignition was detected at < 400°C in a standard A16 study.

8.10.2 Comparison with the CLP criteria

A substance is classified as self-heating when a positive result is obtained in the test method outlined in subsection 33.3.1.6 of the UNRTDG Manual of Tests and Criteria. No data are available, but in an A16 study, no self-ignition was detected at temperatures below 400°C. Further, considering the structure and other available information on the physcio-chemcial properties, there is no evidence that fluxapyroxad possess self-heating properties. Therefore, the criteria for classification are not met.

8.10.3 Conclusion on classification and labelling for self-heating substances

Not classified – conclusive but not sufficient for classification.

8.11 Substances which in contact with water emit flammable gases

Table 11: Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference
EC A.12	No gas evolved on contact with water.		2008/1070100 Loehr S. 2008

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

No gas was evolved on contact of the substance with water.

8.11.2 Comparison with the CLP criteria

Substances which react with water to emit flammable gases are considered for classification in this hazard class. No gas was evolved following contact of fluxapyroxad with water, this is also evident from experience in handling and use. Therefore the classification criteria are not met.

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

Not classified – conclusive but not sufficient for classification.

8.12 Oxidising liquids

Not relevant as the active substance is a solid.

8.13 Oxidising solids

Table 12: Summary table of studies on oxidising solids

Method	**Results**	**Remarks**	**Reference**
EC A.17 Measurement and expert justification (99.4 % pure)	Not oxidizing. The maximum burning rate (2.35 mm/s) was lower than that of the reference material.	-	2008/1070100 Loehr S. 2008

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

The maximum burning rate of the fluxapyroxad test mixture was found to be 2.35 mm/s. This was lower than the maximum burning rate of the reference material.

8.13.2 Comparison with the CLP criteria

A substance is classified as an oxidising solid when the burning time of a sample-to-cellulose mixture is less than or equal to the burning time of the appropriate reference sample. A mixture of fluxapyroxad had a lower burning rate than the reference material. Therefore, the criteria for classification are not met.

8.13.3 Conclusion on classification and labelling for oxidising solids

Not classified – conclusive but not sufficient for classification.

8.14 Organic peroxides

Not relevant as the chemical structure of the active substance does not exhibit a peroxide moiety.

8.15 Corrosive to metals

Table 13: Summary table of studies on the hazard class corrosive to metals

Method	Results	Remarks	Reference
ASTM G31-72	Corrosion rates after exposure for 7 days at 24.7°C (average): Steel 0.0239 mm/year Aluminium 0.0067 mm/year	Used method is similar to procedure described in UN RTDG Manual of test and Criteria.	2009/3000159 Ferreira L. 2009

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

The corrosion rates following exposure for 7 days at 24.7ºC were 0.0239 mm/year in steel and 0.0067 mm/year in aluminium. It is noted that the study employed a lower test temperature than stated in the method provided in section 37.4 of the UN RTDG Manual of Tests and Criteria.

8.15.2 Comparison with the CLP criteria

A substance is classified as corrosive to metals under CLP using the test method outlined in section 37.4 of the UN RTDG Manual of Tests and Criteria. The test method notes that it is used to determine the corrosive properties of 'liquids and solids that may become liquids on transport'. The test conducted on fluxapyroxad was broadly in line with the method provided in the UN RTDG Manual of Tests and Criteria but it employed a lower temperature (24.7ºC as opposed to 55ºC). In this study, the corrosion rates in steel and aluminium were determined to be 0.0239 and 0.0067 mm/year respectively. This is lower than the value of 3.25 mm/year noted in the classification criteria. Further, it is noted that fluxapyroxad is a solid with a melting point of ~157ºC and relatively low water solubility. Taking account of all available information, it is considered that fluxapyroxad is not corrosive to metals.

8.15.3 Conclusion on classification and labelling for corrosive to metals

Not classified – conclusive but not sufficient for classification.

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).

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 14: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Kinetic study in rats OECD 417; GLP Administration via gavage Wistar Rats Principal Design 1) Blood/plasma levels after single administration of 5, 50 or 500 mg/kg bw; blood sampling at intervals up to 168 hours 2) Balance/excretion experiment - single administration of 7.5 or 150 mg/kg and 14 time 12C followed by 1 time 14C Fluxapyroxad; sampling of urine and faces for up to 168 hours 3) Tissue distribution after single doses of 7.5 and 150 mg/kg bw; organ sampling at 4 times depending at estimated Tmax, 1/2 max, T1/4 max, T1/8 max. 4) Excretion via bile: Sampling of bile, urine and faces at intervals up to 72 hours after single oral doses of 7.5 and 150 mg/kg bw	1. Part: Max blood/plasma concentrations after 1, 8, 24 h after administration of 5, 50 and 500 mg/kg bw. Levels comparable between ♂&♀. Linear increase of AUC 2. Part: Almost complete excretion in urine and faces (≥ 90%) with a somewhat higher urinary excretion at the low dose (♂10.5%, ♀16.8%) than at the high dose (♂3.5%, ♀ 9.0%) 3. Part: Residues GIT >>Liver >Kidney>Thyroid. Initially relatively high levels in adipose tissue 4. Part: Recovery from bile: high dose ♂59%, ♀63%; low dose ♂51%, ♀56%. Including recovery from urine systemic absorption was approx. 68% in males and 80% in females at the low dose	None	Anonymous (2009a)
Metabolism study in rats OECD 417; GLP Administration via gavage Wistar Rats Principal design: 1) Identification of metabolites in urine and faeces after a single oral dose of 150 mg/kg bw using the pyrazole and aniline label. 2) Determination of the metabolite pattern and quantification of metabolites in urine, faeces and bile extracts from samples generated in the Fluxapyroxad kinetic study (Anonymous (2009a). 3) Identification and quantification of metabolites in liver, kidney, plasma and fat (150 mg/kg bw) or determination of the metabolite pattern and quantification of metabolites (7.5 mg/kg bw) using the pyrazole label.	Fluxapyroxad is intensively metabolized and conjugated. Main metabolic reactions were the - hydroxylation at the biphenyl ring, - N-demethylation at the pyrazol ring. - loss of fluoride at the biphenyl ring. - and the conjugation with glucuronic acid and glutathione. The cleavage of the amide bond between pyrazole and biphenyl ring is a negligible metabolic pathway in the rat.	None	Anonymous (2009b)

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

The toxicokinetics of Fluxapyroxad have been evaluated in well-conducted, guideline compliant studies investigating the absorption, distribution, metabolism and excretion of Fluxapyroxad in the rat following oral administration of single (low and high) and multiple (low) doses. The studies were conducted using a mixture of radiolabelled Fluxapyroxad, either at the aniline-ring or the 4-position of the pyrazole ring, in addition to ^{15}N-Fluxapyroxad and unlabelled Fluxapyroxad.

Absorption

The pharmacokinetic profile of Fluxapyroxad was determined following a single low, mid or high oral gavage dose (5, 50 or 500 mg/kg bw/day) to male and female rats by measuring the radioactivity present in blood and plasma up to 168 hours post dosing. Peak plasma concentrations displayed a linear relationship with dose level and were comparable between males and females. Additional kinetic parameters determined such as plasma half-life and AUC were also comparable between male and female animals. The results of the kinetic study were used to determine the dose levels required for the ADME studies.

The oral absorption of Fluxapyroxad was estimated following a single low or high oral gavage dose (7.5 or 150mg/kg) to bile-duct cannulated male and female rats by measuring the radioactivity present in urine, bile, cage wash, faeces and carcass up to 168 hours post dosing. Absorption (estimated by adding together the amount of radioactivity in bile, urine and residual carcass) was similar in male and female rats and at both dose levels. Absorption was estimated to be 68-80% and 65-67% of the administered dose by 168 hours post-dosing, for the low and high dose level respectively. The oral absorption of Fluxapyroxad was also estimated in intact animals by measuring the radioactivity present in faeces after a single high oral gavage dose (150 mg/kg bw/day) to male and female rats up to 168 hours post dosing. The proportion of parent compound present in the faeces of male and female rats was comparable and estimated that approximately 75% of the dose had been absorbed.

Distribution

A tissue depletion study showed that following a single oral dose of Fluxapyroxad there was wide distribution of radioactivity, with peak tissue concentrations observed at the first sampling time (1 and 16 hours post dose for low and high dose, respectively). At this time point, the highest mean concentrations were seen in stomach contents, stomach, gut contents, gut, liver and adrenal glands in both sexes at both dose levels. Additionally, high concentrations of radioactivity were seen in the adipose tissue of male and females from the high dose group 16 hours post dosing. Generally, concentrations in all tissues declined with elimination half-lives ranging from 7 to 40 hours with the exception of the concentrations determined in the adipose tissue, gut content and gut in the low dose group which peaked approximately 8 hours post dosing and declined with a subsequent half-life of approximately 20 hours. In the low dose group, at 48 hours post dose, low concentrations of radioactivity approaching background levels were measured in most tissues with the exception of the gut contents. At the high dose level, 96 hours post dosing the level of radioactivity was negligible with the exception of the kidney and the blood cells, which still contained approximately between 9-24% and 19-28% respectively, of the concentration determined at 16 hours post dosing. Seven days after dosing the radioactivity had decreased to between 0.01-0.1% of that measured at 96 hours post dosing.

A study conducted to investigate the blood kinetics of Fluxapyroxad over 168 hours following a single oral administration of either 5, 50 or 500 mg/kg bw/day showed that maximum plasma concentrations (Cmax) of radioactivity were attained (Tmax) at 1, 8 or 24 hours post dose, and declined with a mean terminal elimination half-life of 30.1, 36, and 38.5 (53.2 for males) hours, respectively. Pharmacokinetic parameters of radioactivity in blood were not appreciably different from those in plasma, indicating that there was no selective binding of Fluxapyroxad and/or its metabolites to blood cells. Systemic exposure to Fluxapyroxad and/or its metabolites tended to be greater (1.3-2.5 fold) in females compared to males. The relationship between dose and systemic availability was linear in both sexes.

Metabolism

Fluxapyroxad was extensively metabolised giving rise to up to 51 metabolite types (including conjugates) with potential for multiple isomers in most groups. The major metabolites of biotransformation of Fluxapyroxad are formed by the hydroxylation at the biphenyl ring, N-demethylation at the pyrazole ring, loss of a fluorine atom at the biphenyl ring, and conjugation with glucuronic acid or with glutathione derivatives. An additional, but negligible route of biotransformation is cleavage at the amide bond between the pyrazole ring and the biphenyl ring. Parent Fluxapyroxad was not detected in urine or bile of low, high and repeat dose males and females but accounted for approximately 30% of the radioactivity detected in the faeces of both sexes. Urine and bile contained approximate equal quantities of conjugated and unconjugated metabolites.

Excretion

After absorption, Fluxapyroxad is rapidly distributed and excreted after a single oral dose to male and female rats. The major route of elimination was via the faeces (85- 91% of the administered dose 7 days post dosing), with urinary elimination accounting for 3.4-9.3% of the administered dose. The routes and rates of excretion were similar for males and females independent of dose level, although faecal excretion was slightly higher in the low dose group in males and females. In a repeat dose study, the absorption and elimination was also rapid with the majority of an administered dose being excreted 96 hours post dosing. The major route of elimination was via the faeces (84-86% of a dose), with urinary elimination accounting for 6.8-9.3% of the administered dose. The excretion pattern after multiple dosing is comparable to a single oral administration of 150 mg/kg bw/day and is indicative that Fluxapyroxad is unlikely to accumulate in tissues through repeated exposure.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Table 15: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute toxicity, oral, OECD 423, GLP compliant	Rat, Sprague-Dawley, female, 6 females/group in 2 subgroups of each 3 rat	Fluxapyroxad Purity: 99.4%	2000 mg/kg bw, single oral administration (gavage); vehicle: 0.5% aqueous CMC	LD_{50} > 2000 mg/kg bw	Anonymous (2008a)
Acute toxicity, oral[#] OECD 423, GLP compliant	Rat, Wistar, male, 6 males/group in 2 subgroups of each 3 rats	Fluxapyroxad Purity 99.4%	2000 mg/kg bw, single oral administration (gavage); vehicle: 0.5% aqueous CMC	LD_{50} > 2000 mg/kg bw	Anonymous (2012a)

[#]**Study in male rats requested by Chinese Authorities**

Table 16: Summary table of other studies relevant for acute oral toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Neurotoxicity (single dose with 14-day observation period OECD 424 CLP compliant	Fluxapyroxad Purity: 99.4%	Rat, Wistar, male and female 10 animals/sex/ group 0, 125, 500, 2000 mg/kg, single oral administration (gavage); vehicle: 0.5% aqueous CMC	No mortality occurred. One 125 mg/kg male displayed skin lesions at both forelimbs starting study day 7 (considered incidental). FOB at Day 0: slightly increased landing foot-splay (males, 2000 mg/kg), reduced rearing (≥ 500 mg/kg, males) and decreased motor activity (2000 mg/kg, both sexes). No findings at later times.	Anonymous (2009c)

10.1.1 Acute toxicity

In OECD 423 studies a starting dose of 2000 mg/kg bw was given to 2 groups of 3 fasted female (study 1) or males rats (study 2) by oral gavage. No mortality occurred in either sex. Clinical signs consisted of hypoactivity (3 females), piloerection (2 females, 6 males), dyspnoea (6 males) and impaired general state (6 males). All animals were free of clinical signs latest the day after administration. No macropathological findings were noted at the end of the 14-day observation period.

No mortality was observed in groups of 10 male and female rats after single oral doses up to 2000 mg/kg in an acute neurotoxicity study.

10.1.2 Comparison with the CLP criteria

The oral LD_{50} of > 2000 mg/kg bw for rats is above the value for classification provided in the Regulation (i.e. 2000 mg/kg bw).

10.1.3 Conclusion on classification and labelling for acute oral toxicity.

Not classified – Conclusive but not sufficient for classification

10.2 Acute toxicity - dermal route

Table 17: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
Acute toxicity, dermal, OECD 402, GLP compliant	Rat, Sprague-Dawley, male/female, 5/sex/group	Fluxapyroxad Purity: 99.4%	2000 mg/kg bw, single dermal application (semi-occlusive), 24 h exposure	LD_{50} > 2000 mg/kg bw	Anonymous (2008b)

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

In an acute dermal toxicity study five Sprague-Dawley rats per sex were treated for 24 h under semi-occlusive conditions with the limit dose of 2000 mg/kg Fluxapyroxad. No mortality occurred and no clinical signs of toxicity were observed. Signs of local irritation (erythema in 2 male and 2 female rats) were noted on day 2, only. In conclusion, the dermal LD_{50} was >2000 mg/kg bw.

10.2.2 Comparison with the CLP criteria

The dermal LD_{50} of > 2000 mg/kg bw for rats is above the value for classification provided in the Regulation (i.e. 2000 mg/kg bw). No classification for acute dermal toxicity is proposed.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Not classified – Conclusive but not sufficient for classification

10.3 Acute toxicity - inhalation

Table 18: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, , form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Acute toxicity, inhalation OECD 403, GLP compliant	Rat, Wistar, male/female, 5/sex/group	Fluxapyroxad Purity: 98.9% dust MMAD: 3.3/3.4 µm GSD: 2.1/2.2	5.1 mg/L, head-nose inhalation, 4 h exposure	LC_{50} > 5.1 mg/L	Anonymous (2008c)

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

In an OECD 403 acute inhalation study groups of 5 Wistar rats/sex/dose toxicity data 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. Clinical signs of toxicity included visually increased respiration (all animals), abdominal respiration (all animals), piloerection (all animals), and squatting posture (2 males and 4 females). The clinical signs started on the day of administration and were fully reversible within 7 days. Macroscopic examination at the end of the observation period revealed no apparent abnormalities. In conclusion, the inhalation LC_{50} was > 5.1 mg/L.

10.3.2 Comparison with the CLP criteria

The 4 h inhalation LC_{50} of > 5 mg/L for rats is above the value for classification in the CLP Regulation (i.e. 5 mg/L dust/mist). No classification for acute inhalation toxicity is proposed.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Not classified – Conclusive but not sufficient for classification

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**.

10.4 Skin corrosion/irritation

Table 19: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
Acute dermal irritation/corrosion, OECD 404, GLP compliant	Rabbit, New Zealand White, 2 male, 1 female	Fluxapyroxad Purity: 99.96%	0.5 g moistened with tap water single topical application (semi-occlusive), 4 h exposure	Grade 1 erythema 1 h after patch removal (all animals) Average individual scores 24 to 72 h: - erythema: 0.0, 0.0, 0.7 - oedema: 0.0, 0.0, 0.0 - fully reversible within 72 h	Anonymous (2006a) Amendment: Anonymous (2008d)

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

In an OECD 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. Grade 1 erythema was noted 1 h after patch removal. 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.

10.4.2 Comparison with the CLP criteria

No corrosion of the skin occurred. The mean scores for erythema/eschar or oedema formation were < 2.3 in all animals. The signs of dermal irritation resolved within 72 h at the latest, i.e. prior to the end of the 14 day observation period. The criteria for classification are not met.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Not classified – Conclusive but not sufficient for classification

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**.

10.5 Serious eye damage/eye irritation

Table 20: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
Acute eye irritation, OECD 405, GLP compliant	Rabbit, New Zealand White, 1 male, 2 females	Fluxapyroxad Purity: 99.7%	0.1 mL (~ 26 mg), instillation into conjunctival sac of the right eye 24 h exposure (rinsing after 24 h)	slight (Grade 1) conjunctival redness in all three animals 1 h after instillation no signs of ocular irritation (corneal opacity, iris inflammation, conjunctival redness or chemosis) in any animal 24, 48 and 72 h after administration Mean scores (24-72 h): - opacity: 0.0, 0.0, 0.0	Anonymous (2008e)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
				- iris: 0.0, 0.0, 0.0 - conj. redness: 0.0, 0.0, 0.0 - conj. chemosis: 0.0, 0.0, 0.0	
Acute eye irritation[#] OECD 405, GLP compliant	Rabbit, New Zealand White, 3 males	Fluxapyroxad Purity: 99.7%	0.1 mL (~ 21-6 mg) single instillation of undiluted test substance, 24 h exposure (rinsing after 24 h)	slight (Grade 1) conjunctival redness in one animal 1 h after instillation no signs of ocular irritation (corneal opacity, iris inflammation, conjunctival redness or chemosis) in any animal 24, 48 and 72 h after administration Mean scores (24-72 h): - opacity: 0.0, 0.0, 0.0 - iris: 0.0, 0.0, 0.0 - conj. redness: 0.0, 0.0, 0.0 - conj. chemosis: 0.0, 0.0, 0.0	Anonymous (2012b)

[#]Repeat of initial study requested by Brazilian Authorities due to the lack of using Fluorescein during eye examination

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

In two OECD 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. Slight redness (grade 1) was observed in 3/3 animals (study 1) and 1/3 animals (study 2) one hour after instillation. This was fully reversible within 24 h. No other reactions were observed in the eye of the animals throughout the whole study period. The mean scores over 24, 48 and 72 h were 0.0 for corneal opacity, iris inflammation, conjunctival redness, and chemosis. In conclusion, no eye irritation was observed after treatment with Fluxapyroxad.

10.5.2 Comparison with the CLP 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). In conclusion, the criteria for classification are not met.

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

Not classified – Conclusive but not sufficient for classification

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**.

10.6 Respiratory sensitisation

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

No data.

10.6.2 Comparison with the CLP criteria

The potential of Fluxapyroxad to cause respiratory sensitisation was not investigated directly, as there are no recognised and validated animal tests for respiratory sensitisation. However, given that Fluxapyroxad does not require classification for skin sensitisation and no indication of respiratory sensitisation is available from observations in humans, Fluxapyroxad is considered unlikely to be a respiratory sensitiser.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

Not classified – No Data Available

10.7 Skin sensitisation

Table 21: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
Guinea Pig Maximization assay OECD 406, GLP compliant	Guinea pig, Dunkin Hartley, females, 10 in control group, 20 in test group	Fluxapyroxad, 99.7%	Intradermal induction: 5% (in 1% aqueous CMC) Topical induction: 60% (in 1% aqueous CMC, 48 h occlusive) Topical challenge: 25% (in 1% aqueous CMC, 24 h occlusive)	Challenge results: Vehicle control group: 1/10 (erythema, score 2 after 24 h) 1/10 (erythema, score 1 after 48 h) Test group: 2/20 (erythema, score 1 after 24 h) 1/20 (erythema, score 1 after 48 h) **Not sensitising**	Anonymous (2008f)

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

In a guideline Guinea pig maximisation study Fluxapyroxad was assessed for its potential to cause skin sensitisation. Twenty Guinea pigs were intradermally induced by injection with 5% test material in 1% aqueous CMC. Topical application was performed after 7 days, using 48 h occlusive applications of Fluxapyroxad (60% in 1% aqueous CMC). After a further two weeks, the animals were challenged using a 24 hour occlusive application of Fluxapyroxad (25% in 1% aqueous CMS). Skin reactions were observed in 1 control and in 2 (10%) test group animals.

10.7.2 Comparison with the CLP criteria

A response in 30% of the animals in an adjuvant test is required for classification. Two out of 20 (i.e., 10%) displayed skin reactions after challenge with fluxapyroxad. Therefore, the criteria for classification are not met.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Not classified – Conclusive but not sufficient for classification

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**.

10.8 Germ cell mutagenicity

Table 22: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Bacterial reverse mutation assay (Ames test) OECD TG 471 (adopted 1997) GLP compliant	Fluxapyroxad, purity: 99.4%	*S.typhimurium* strains TA 1535, TA 1537, TA 98, TA 100; *E.coli* strain WP2 uvrA; plate incorporation and pre-incubation assay; with/without S-9 mix 20 - 5000 µg/plate dose selection based on guideline requirements	**Negative ± S9** Cytotoxicity and precipitation at ≥ 500 µg/mL	Schulz M., Landsiedel R. (2008e) BASF DocID 2008/1028479
In-vitro chromosome aberration assay in mammalian cells OECD TG 473 (adopted 1997) GLP compliant	Fluxapyroxad, purity: 99.7%	Chinese Hamster V79 cells; with/without S-9 mix 3.1 – 400 µg/mL Experiment 1: 4 h exposure, sampling after 18 h (i.e. 14 h recovery) -/+ S9 400 to 6.3 µg/ml[a] spaced by a factor of 2 Experiment 2a: 18 h exposure, 0 h recovery; –S9 100 to 3.1 µg/ml[b] spaced by a factor of 2 Experiment 2b: 18 h exposure, 10 h recovery (i.e. sampling after 28 h); - S9 100 to 12.5 µg/ml[b] spaced by a factor or 2 Experiment 2c: 4 h exposure, 24 h recovery (i.e. sampling after 28h); + S9 100 to 3.1 µg/ml[b] spaced by a factor of 2 — [a] dose selection based on cytotoxicity test performed during HPRT test (Chinese Hamster K1 cells) [b)] dose selection based on cytotoxicity at ≥ 100 µg/ml in Experiment 1	**Negative ± S9** Cytotoxicity at ≥ 100 µg/mL Experiment 1: there was no increase in the incidence of structurally or numerically aberrant cells with and without S9 Experiment 2a and 2b: there was no increase in the incidence of structurally or numerically aberrant cells without S9 Experiment 2c: At the lowest concentration scored (12.5 µg/ml) a slightly increased number of aberrant cells (7.0% vs. 2.5% in controls; HCD 0 – 5.0%)) was noted. Due to the missing dose response this is considered to be of no toxicological relevance.	Schulz M., Landsiedel R. (2008b) BASF DocID 2007/1023153
In-vitro forward mutation assay in mammalian cells (HPRT test) OECD TG 476 (adopted 1997) GLP compliant	Fluxapyroxad, purity: 99.7%	CHO-K1 cells; with/without S-9 mix 5 – 100 µg/mL; vehicle DMSO (may 1% in culture media) Preliminary cytotoxicity test: Relative cloning efficiency between 1 and 11% at 100 µg/mL Experiment 1:	**Negative ± S9** Experiment 1: Mutant frequency without S9 was lower than in vehicle control, with S9 exceeded the vehicle control by up to 1.5-fold but was not statistically significant and within HCD	Schulz M., Landsiedel R. (2007a) BASF DocID 2007/1020715

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		4 h w/wo S9; 5.0, 10.0, 20.0, 50.0, and 100.0 µg/mL Experiment 2a: 24 h -S9; 6.3, 12.5. 25, 50, 75 and 100 µg/mL Experiment 2b: 4h + S9; 6.3, 12.5. 25, 50, 75 and 100 µg/mL	Experiment 2a: Mutant frequency max. 2.4-fold higher than control (n.s., not dose dependent, within HCD) Experiment 2b: Mutant frequency max. 1.4 fold higher than control (n.s., not dose related, within HCD).	

Table 23: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
In-vivo micronucleus test OECD TG 474 (adopted 1997) GLP compliant	Fluxapyroxad, purity: 99.6%	Male NMRI mouse (oral gavage, twice within 24h; vehicle: corn oil) 500, 1000, 2000 mg/kg bw Preliminary toxicity test with 2000 mg/kg bw: no mortality, no clinical signs of toxicity (m/f) – as no difference between sexes were observed, the main study was performed in male mice	**Negative** (not aneugenic/clastogenic) PCE/NCE ratio not affected No signs of systemic toxicity (systemic distribution assumed based on effects observed in chronic mouse study) At the low dose of 500 mg/kg a significantly higher number of micronuclei were noted (1.7‰ vs. 1.2‰). The absence of a dose response relationship and the fact that the incidence was within the HCD (0.3 to 2.2‰) indicated the incidental character of the finding. The positive controls Vincristine sulphate for aneugenic effects and Cyclophosphamide for clastogenic effects resulted in 36.2‰ and 12.8‰ micronucleated polychromatic erythrocytes. The former included 9.8‰ large micronuclei. No direct proof of the exposure of the bone marrow to the test item was provided. However, based on the results of plasmakinetic study in another rodent species (rat) an exposure of the bone marrow can be reasonably assumed.	Anonymous (2006b)

In-vivo Unscheduled DNA Synthesis (UDS) OECD TG 486 (adopted 1997) GLP compliant	Fluxapyroxad, purity: 99.7%	Wistar rat, male (single oral gavage); Preparation of hepatocytes from groups of 3 rats 3 or 14 h after exposure (1000, 2000 mg/kg bw Fluxapyroxad; 50 mg/kg bw positive control 2-AAF) Vehicle: corn oil Preliminary toxicity test with 2000 mg/kg bw: no mortality no clinical signs of toxicity except apathy (m/f) – as no difference between sexes were observed, the main study was performed in male rats	**Negative** (no increase in UDS in rat hepatocytes) No signs of systemic toxicity observed except apathy at 2000 mg/kg bw (systemic distribution assumed based on effects observed in several short and long term rat studies) No increase of the mean net nuclear grain (NNG) count in Fluxapyroxad treated rat hepatocytes at either dose. Positive control 2-AAF resulted in mean NNG counts of about 8.3 and 75% of the scored cells were in repair.	Anonymous (2008g), Amendment: Anonymous (2008h)

10.8.1 Summary of germ cell mutagenicity

The bacterial (Ames) and mammalian gene mutation assays (HPRT in Chinese Hamster ovary K1 cells) resulted in negative outcome in presence and absence of S9. Likewise, the *in-vitro* chromosome aberration assay in Chinese Hamster V79 cells did not indicate a clastogenic or aneugenic activity of Fluxapyroxad.

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. Even though no decline of PCEs in the bone marrow was observed, data from toxicokinetic study in another rodent species (rat) indicates that Fluxapyroxad and/or its metabolites reaches the blood/bone marrow.

No studies on germ cells were performed, which is justified based on the negative effects of Fluxapyroxad in *in-vitro* studies and *in-vivo* mutagenicity studies in somatic cells.
There is no human information or other relevant information on potential genotoxic activity of Fluxapyroxad.

10.8.2 Comparison with the CLP criteria

Under CLP, substances can be classified as a Cat 1A or 1B or Cat 2. The criteria for a classification are given below.

Classification in Cat 1B is based on:

- positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or
- positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells.
- positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny.

There are no human data or positive results *in vivo* studies in somatic cells of mammals other than humans that suggest that Fluxapyroxad causes heritable mutations. Thus, no classification in Cat 1A or Cat 1B is warranted.

Classification in Cat 2 is based on:
Positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from:

- Somatic cell mutagenicity tests *in vivo*, in mammals; or
- other *in vivo* somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.

As no evidence for Fluxapyroxad genotoxicity in somatic cells *in-vivo* or for *in-vitro* genotoxicity no classification in Cat 2 is warranted.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Not classified – Conclusive but not sufficient for classification

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**.

10.9 Carcinogenicity

The carcinogenic potential and chronic toxicity of fluxapyroxad has been well investigated in standard studies in rats and mice (refer to table 24). Additional studies have been conducted to investigate Mode of Action (MoA) and human health relevance. These are summarised and discussed in section 10.9.3 – 10.9.4.

Table 24: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** (* denotes statistically significant change)	**Reference**
2-yr chronic toxicity/ carcinogenicity study OECD 453 GLP compliant Oral. dietary Rat, Han Wistar Chronic toxicity phase: 10/sex/group Carcinogenicity phase: 50/sex/group	Fluxapyroxad Purity: ≥99.2% 0, 50, 250, 1500, 3000 ppm 2.1, 11, 68, and 145 mg/kg bw/d for males; 2.7, 14, 82, and 182 mg/kg bw/d for females Exposure duration Chronic toxicity phase: 52 weeks Carcinogenicity phase: 104 weeks	**Non-neoplastic findings** Mortality in chronic toxicity (one high dose male died) and carcinogenicity group not affected by treatment. Mortality: 12, 16, 12, 10 and 18% in males and 26, 20, 18, 30 and 22% in females at 0, 50, 250, 1500 and 3000 ppm, respectively. **3000 ppm (145/182 mg/kg bw/d in m/f):** Clinical signs of toxicity: Higher incidence of teeth whitening (10m/3f). Body weight development: ↓ bw 10%/23% (m/f)* (at day 728), ↓ bw gain 14%/35% (m/f)* (at day 728) Organ weights (carcinogenicity group): ↑ rel epididymides/testes wt (m*), rel adrenal/brain/heart wt (m/f)*, ↑ rel kidney wt (m*/f); ↑ uterus wt (f*), ↑ abs liver wt 45%/19% (m/f)*; ↑ rel liver wt 61%/55% (m/f)*, ↓ abs spleen wt 9%/26% (m/f)* Macropathology findings: Liver cyst (m); discoloration, enlarged, focus, mass, acinar pattern (m/f); Thyroid: enlarged, mass (m), Skull bones & incisors: discoloration (m/f)) Histopathological findings: Liver: hepatocellular hypertrophy, centrilobular (zone 3); pigment storage, diffuse (m/f)*; Spongiosis hepatis; basophilic foci - NOS (m*); eosinophilic foci (f*); Thyroid: hyperplasia, follicular (m*/f); altered colloid (m/f)*; Femur: Deposition of Perl´s Prussian blue positive material (m/f)*; Skull bones: hyperostosis. **1500 ppm (68/82 mg/kg bw/d in m/f):** Clinical signs of toxicity: Higher incidence of teeth whitening (17m/24f). Body weight development ↓ bw 3%/16% (m/f*) (at day 728); ↓ bw gain 4%/25% (m/f*) (at day 728) Altered haematology and clinical chemistry parameters: ↓ PTT (m/f)*, ↓ MCV, MCH (f*); HCT (m*); ↓ ASAT (f*), Cl^-, tBil (m/f)*; ↑ SGGT (m*), Ca^{2+}, tProt, Chol (m/f)*. Organ weights (carcinogenicity group):↑ rel epididymides/testes wt (m*), rel brain/heart wt (f*), ↑ rel kidney wt (m/f); ↑ uterus wt (f*); ↑ abs liver wt 33%/13% (m/f)*; ↑ rel liver wt 38%/36% (m/f)*; ↓ abs spleen wt 9% (f*) Macropathology findings: Liver: cyst (m), discoloration, focus, mass, acinar pattern (m/f); Thyroid: enlarged, mass (m); Incisors: discoloration (m/f).	Anonymous (2009d)

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results (* denotes statistically significant change)</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
Histopathological findings: Femur: Deposition of Perl´s Prussian blue positive material (m/f)*
Liver: hypertrophy, centrilobular (zone 3); pigment storage, diffuse (m/f)*; Spongiosis hepatis (m*); eosinophilic foci (f); Thyroid: hyperplasia, follicular (m/f)*; altered colloid (m/f)*
250 ppm (11/14 mg/kg bw/d in m/f):
Body weight development ↓ bw 12% (f*) (at day 728); ↓ bw gain 19% (f*) (at day 728).
Altered haematology and clinical chemistry parameters: ↓ PTT (f*); ↓ tBil (m/f)*; ↑ Chol (m/f)*; Glob. (f*)
Organ weights (carcinogenicity group): ↑ rel brain/heart/uterus wt (f*); ↑ abs kidney wt (m*/f); ↑ rel kidney wt (f*); ↑ abs liver wt 11% (m*); ↑ rel liver wt 8%/12% (m/f)*; ↓ abs spleen wt (f*)
Macropathology findings: Liver: mass (m) (focus; m/f)
Histopathological findings: Femur: Deposition of Perl´s Prussian blue positive material (m/f)*; Liver: hepatocellular hypertrophy, centrilobular (zone 3) (m/f)*; eosinophilic foci (f*)
50 ppm (2.1/2.7 mg/kg bw/d in m/f):
No treatment-related effects
Neoplastic findings
Liver
Primary neoplastic findings at necropsy (Incidence)
<table>
<tr><td>Parameter</td><td colspan="5">Dietary concentration of fluxapyroxad (ppm)</td></tr>
<tr><td></td><td colspan="5">Males/Females</td></tr>
<tr><td></td><td>0</td><td>50</td><td>250</td><td>1500</td><td>3000</td></tr>
<tr><td>Organs exam.</td><td>50</td><td>50</td><td>50</td><td>50</td><td>50</td></tr>
<tr><td colspan="6">Hepatocellular tumours - males</td></tr>
<tr><td>- Adenoma</td><td>0</td><td>0</td><td>4 (8%)</td><td>7** (14%)</td><td>15** (30%)</td></tr>
<tr><td>- Carcinoma</td><td>1 (2%)</td><td>0</td><td>1 (2%)</td><td>3 (6%)</td><td>9 (18%)**</td></tr>
<tr><td colspan="6">Hepatocellular tumours - females</td></tr>
<tr><td>- Adenoma</td><td>0</td><td>2 (4)</td><td>0</td><td>4 ** (8%)</td><td>7** (14*)</td></tr>
<tr><td>- Carcinoma</td><td>1 (2%)</td><td>1 (2%)</td><td>0</td><td>0</td><td>0</td></tr>
</table>
Historical control data hepatocellular tumours (% incidence)
8 Studies conducted between 08.1999 and 01.2008
males, adenoma: mean: 2.0%, min: 0.0%, max. 4.0%
males, carcinoma: mean: 1.5%, min: 0.0%. max. 6.0%
females, adenoma: mean: 0.8%, min: 0.0%, max. 6.0%
females, carcinoma: mean: 1.8%, min: 0.0%. max. 6.0%
</td><td></td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results (* denotes statistically significant change)</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
<u>Thyroid</u>

Primary neoplastic findings at necropsy (Incidence)
<table>
<tr><td rowspan="2">Parameter</td><td colspan="5">Dietary concentration of fluxapyroxad (ppm)</td></tr>
<tr><td colspan="5">Males/Females</td></tr>
<tr><td></td><td>0</td><td>50</td><td>250</td><td>1500</td><td>3000</td></tr>
<tr><td>Organs exam.</td><td>50/50</td><td>50/49</td><td>50/50</td><td>50/48</td><td>50/50</td></tr>
<tr><td colspan="6">C-cell tumours - males</td></tr>
<tr><td>- Adenoma</td><td>5 (10%)</td><td>6 (12%)</td><td>4 (8%)</td><td>2 (4%)</td><td>6 (12%)</td></tr>
<tr><td>- Carcinoma</td><td>1 (2%)</td><td>0</td><td>0</td><td>0</td><td>1 (2%)</td></tr>
<tr><td colspan="6">Follicular cell tumours Males</td></tr>
<tr><td>- Adenoma</td><td>3 (6%)</td><td>2 (4%)</td><td>4 (8%)</td><td>8 (16%)</td><td>9 (18%)</td></tr>
<tr><td>- Carcinoma</td><td>0</td><td>0</td><td>1 (2%)</td><td>1 (2%)</td><td>3 (6%)</td></tr>
<tr><td colspan="6">C-cell tumours - females</td></tr>
<tr><td>- Adenoma</td><td>13 (26%)</td><td>6 (12%)</td><td>6 (12%)</td><td>5 (10%)</td><td>8 (16%)</td></tr>
<tr><td>- Carcinoma</td><td>0</td><td>1 (2%)</td><td>0</td><td>0</td><td>1 (2%)</td></tr>
<tr><td colspan="6">Follicular cell tumours Females</td></tr>
<tr><td>- Adenoma</td><td>0</td><td>3 (6%)</td><td>1 (2%)</td><td>3 (6%)</td><td>2 (4%)</td></tr>
<tr><td>- Carcinoma</td><td>2 (4%)</td><td>0</td><td>1 (2%)</td><td>0</td><td>1 (2%)</td></tr>
</table>

<u>Historical control data follicular cell tumours (% incidence)</u>
8 Studies conducted between 08.1999 and 01.2008

males, adenoma: mean: 13.0%, min: 4.0%, max. 28.0%
males, carcinoma: mean: 2.3%, min: 0.0%. max. 4.0%

females, adenoma: mean: 2.0%, min: 0.0%, max. 4.0%
females, carcinoma: mean: 1.5%, min: 0.0%. max. 6.0%

NOAEL (syst. toxicity): 50 ppm (2.1/2.7 mg/kg bw/d in m/f)§

NOAEL (carcinogenicity): 50 ppm (2.1/2.7 mg/kg bw/d in m/f)§

LOAEL (toxicity/carcinogenicity): 250 ppm (11/14 mg/kg bw/d in m/f)
</td><td></td></tr>
<tr><td>18-months carcinogenicity study
OECD 451
GLP
Dietary
Mouse, C57BL/6J Rj
Interim sacrifice group: 10/sex
Carcinogenicity group (18 m): 50/sex</td><td>Fluxapyroxad
Purity: ≥99.2%
0, 150, 750, 3000, 6000 ppm
Carcinogenicity group (18 mth) 21, 107, 468, 996 mg/kg bw/d for males; 33, 158, 652, 1307 mg/kg bw/d for females
Interim sacrifice group (9 mths), 6000 ppm: 1119/ 1512 mg/kg bw/d for</td><td>
Non-neoplastic findings

Mortality in carcinogenicity group not affected by treatment. Mortality: 18, 10, 20, 20 and 12% in males and 22, 16, 26, 18 and 6% in females at 0, 150, 750, 3000 and 6000 ppm, respectively.

6000 ppm (996/1307 mg/kg bw/d in m/f):

Clinical signs of toxicity: Higher incidence of teeth whitening, all animals affected (m/f)

Body weight development: ↓ bw (m*) up to -17.5%, (f*) transiently up to -12.2%; ↓ bw gain (m*) – up to -78%, (f*) up to -27%

Clin. chemistry: ↓ Chol (m/f)*; TRIG (m*/f), ↑ ALP (f*) (investigated only after 9 months in satellite group)

Organ weight: ↑ abs kidney wt (m*); ↑ abs liver wt 24%/18% (m/f)*; ↑ rel liver wt 32%/16% (m/f)*; ↓ abs spleen wt 26% (m*)

Macropathology findings: <u>incisor</u> (maxilla & mandible) discoloured; <u>liver</u>: enlarged (m), focus (m)

Histopathology findings: <u>Liver:</u> hepatocellular hypertrophy, central (m*) 24/50; hepatocellular hypertrophy, peripheral (f*) 47/50; fatty
</td><td>Anonymous (2009e)
Amendment: Anonymous (2010a)</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results (* denotes statistically significant change)</th><th>Reference</th></tr>
<tr><td></td><td>m/f</td><td>
change – macrovesicular (or steatosis) (m/f)*
3000 ppm (468/652 mg/kg bw/d in m/f):
Clinical signs of toxicity: Higher incidence of Teeth whitening (m (22/59) / f (20/50))
Body weight development: ↓ bw (m*) up to -12%; ↓ bw gain (m*) up to -27%
Organ weighs :↑ abs kidney wt (m*); ↑ abs liver wt 22%/9% (m/f)*; ↑ rel liver wt 26%/7% (m*/f); ↓ abs spleen wt 25% (m*)
Macropathology: <u>incisor</u> (maxilla & mandible) discoloured
Histopathology findings: <u>Liver:</u> hypertrophy, peripheral (f*) 15/50; fatty change, macrovesicular (m/f)*
750 ppm (107/158 mg/kg bw/d in m/f):
Organ weights: ↑ abs liver wt 14%/2% (m*/f); ↑ rel liver wt 10%/7% (m*/f)
Histopathology findings: <u>Liver:</u> fatty change, macrovesicular (m/f)*
150 ppm (21/33 mg/kg bw/d in m/f):
Organ weights: ↑ abs liver wt 15% (m*); rel liver wt 11% (m)
<u>Neoplastic findings:</u>
There were no treatment-related increases in the incidence of tumours in any particular organ or tissue. As there was a treatment related increase in liver tumours in the rat study, the mouse liver tumours are shown below:
<u>Liver</u>
Primary neoplastic findings at necropsy (Incidence)
<table>
<tr><td rowspan="2">Parameter</td><td colspan="5">Dietary concentration of fluxapyroxad (ppm)</td></tr>
<tr><td colspan="5">Males/Females</td></tr>
<tr><td></td><td>0</td><td>150</td><td>750</td><td>3000</td><td>6000</td></tr>
<tr><td>Organs exam.</td><td>50</td><td>50</td><td>50</td><td>50</td><td>50</td></tr>
<tr><td colspan="6">Hepatocellular tumours - Males</td></tr>
<tr><td>- Adenoma</td><td>0</td><td>3</td><td>1</td><td>1</td><td>2</td></tr>
<tr><td>- Carcinoma</td><td>1</td><td>3</td><td>1</td><td>3</td><td>3</td></tr>
<tr><td colspan="6">Hepatocellular tumours - Females</td></tr>
<tr><td>- Adenoma</td><td>0</td><td>0</td><td>0</td><td>2</td><td>3</td></tr>
<tr><td>- Carcinoma</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
</table>

<u>Historical control data hepatocellular tumours (% incidence)</u>
8 Studies conducted between 08.1999 and 01.2008
males, adenoma: mean: 2.0%, min: 0.0%, max. 4.0%
males, carcinoma: mean: 1.5%, min: 0.0%. max. 6.0%
females, adenoma: mean: 0.9%, min: 0.0%, max. 6.0%
females, carcinoma: mean: 0.9%, min: 0.0%. max. 2.0%
NOAEL (toxicity): < 150 ppm (m); 150 ppm (33 mg/kg bw/d in f)
</td><td></td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** (* denotes statistically significant change)	**Reference**
		LOAEL (toxicity): 150 ppm (21 mg/kg bw/d in m)	

** Indicates statistically significant, $p \leq 0.01$ (Wilcoxon test)

10.9.1 Carcinogenicity - Non Human Information

Rat

In a rat carcinogenicity study (Anonymous (2009d)) treatment with Fluxapyroxad did not affect the survival of rats up to the highest dose. General toxicity displayed by reduced body weight and body weight gain was observed in males at 3000 ppm and in females at ≥250 ppm. The liver, thyroid and bones were identified as target organs. In the femur an increased incidence and severity of ferric iron [Fe^{3+}] deposition was noted at dose levels ≥ 250 ppm. Comparable incidences and severity of this finding were noted in all rat studies starting from 28-day to the 1-year chronic toxicity phase of the combined chronic toxicity and carcinogenicity study, indicating no progression of this finding over time. In contrast, hyperostosis of the skull bones was only observed at the top dose in animals killed at terminal sacrifice in the lifetime study.

Non-neoplastic findings in the thyroid consisted of altered follicular cell colloid and follicular hypertrophy/hyperplasia which was predominantly observed in male rats at the end of the 1-year chronic toxicity period as well as in carcinogenicity group animals. The liver as the main target organ was identified by a number of liver related clinical chemistry changes (increased serum γ-glutamyl transferase, total protein, albumin, triglyceride and cholesterol levels as well as decreased total bilirubin and glucose levels), increased liver weights as well as non-neoplastic changes (centrilobular hypertrophy, spongiosis hepatis, increased incidence of certain types of 'foci of cellular alteration' as well as diffuse pigment storage) partially down to the 250 ppm dose level.

Regarding neoplastic findings there were no treatment-related findings at the 12-month interim sacrifice.

Liver Tumours

In the liver an increased incidence of hepatocellular adenoma and carcinoma was noted in males at ≥ 250 ppm (adenoma: 0, 0, 4, 7 and 15 out of 50 animals or 0, 0, 8, 14 and 30%; carcinoma: 1, 0, 1, 3 and 9 out of 50 animals or 2, 0, 2, 6 and 18% at 50, 250, 1500 and 3000 ppm, respectively). In females an increased incidence was noted at ≥ 1500 ppm (adenoma: 0, 2, 0, 4 and 7 out of 50 animals or 0, 4, 0, 8 and 14%; carcinoma: 1, 1, 0, 0 and 0 out of 50 animals or 2, 2, 0, 0 and 0% at 50, 250, 1500 and 3000 ppm, respectively). In males the incidences were above the historical control range at ≥250 ppm (adenoma: 0 - 4%; carcinoma: 0 - 6%). In females the historical control range for adenoma was exceeded at ≥ 1500 ppm (adenoma: 0 - 6%). In conclusion, Fluxapyroxad induced an increased incidence of liver tumours, in male and female rats at study termination.

Thyroid Tumours

A slightly increased number of thyroid follicular cell adenoma (3, 2, 4, 8 and 9 out of 50 animals or 6, 4, 8, 16 and 18% at 0, 50, 250, 1500 and 3000 ppm, respectively) and carcinoma (0, 0, 1, 1 and 3 out of 50 animals or 0, 0, 2, 2 and 6% at 0, 50, 250, 1500 and 3000 ppm, respectively) was noted in males at 1500 ppm and 3000 ppm. While the number of follicular cell adenoma (18%) 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%, respectively 0/50 to 2/50) by one case. The incidence of follicular cell tumours in females (adenoma: 0, 3, 1, 3, 2 or 0, 6, 2, 6, 4%; carcinoma: 2, 0, 1, 0, 1, or 4, 0, 2, 0, 2% at 0, 50, 250, 1500 and 3000 ppm, respectively) was within the historical control range (adenoma; 0-4%; carcinoma 0-6%) and not indicative of a relation to treatment. The incidence of thyroid C-cell adenoma and carcinoma was comparable between control and treated males. In females the highest incidence of C-cell adenoma was noted in control animals. One single C-cell carcinoma was noted in a low dose female.

Mouse

In the dietary study (Anonymous (2009e)) in mice over 18 months, treatment did not adversely affect survival of the animals as the number of decedents was 9, 5, 10, 10 and 6 in males and 11, 8, 13, 9 and 3 in females at 0, 150, 750, 3000 and 6000 ppm, respectively. Systemic toxicity was observed as indicated by effects on body weight development in males at ≥3000 ppm and females at 6000 ppm. The liver was identified as the only target organ as indicated by increases of relative and/or absolute liver weights in males at ≥750 ppm and in females at ≥3000 ppm. Histopathological examination of the liver revealed mainly periportal (males; Zone 3) or peripheral (females; Zone 1) hepatocellular hypertrophy at 6000 ppm and ≥3000 ppm, respectively. In addition, an increased severity and/or incidence of macrovesicular fatty changes in hepatocytes was observed at dose levels ≥750 ppm.

There were no neoplastic findings in the animals killed at 9 months. In the animals killed at 18 months, the overall incidence of neoplastic findings in the treated groups was similar to controls. 21, 25, 18, 17 and 16 out of 50 males and 25, 19, 17, 29 and 28 out of 50 females displayed neoplasms at 0, 150, 750, 3000 and 6000 ppm. The incidence of hepatocellular adenoma (males: 0, 3, 1, 1, 2; females: 0, 0, 0, 2, 3), carcinoma (males: 1, 3, 1, 3, 3; females: 1, 0, 0, 0, 0 appeared to be higher than in controls (in females), however, did not display a dose-response relationship in males and were within the historical control range in females. These changes are considered to be of spontaneous origin, not treatment-related. In conclusion, the mouse carcinogenicity study did not provide evidence for carcinogenic potential.

Conclusion

Overall fluxapyroxad induced liver tumours in male and female rats, with the tumour incidence in males being much greater than in females. Fluxapyroxad also induced a slight increase in thyroid follicular cell adenomas in male rats in a lifetime dietary study along with an increase in thyroid follicular cell carcinomas. No other increases in tumour incidence were noted in rats.

No treatment-related increases in tumour incidence were observed in a lifetime dietary study in mice.

10.9.2 Human information

No relevant information is available.

10.9.3 Other relevant information

Mechanistic studies relevant for findings in the liver

There are various possible mechanistic explanations that can be considered for the weak carcinogenic response in rats, including:

Genotoxicity
Cytoxicity
PPARα receptor activation
CAR/PXR receptor activation
AhR receptor activation
Porphyria
Endocrine
Immunosuppression

The applicant sponsored a series of mechanistic studies (Sections 10.9.3.1-10.9.3.6) to investigate a possible non-genotoxic mode of action involving hepatocyte proliferation, induced via constitutive androstane receptor (CAR) and/or pregnane X receptor (PXR) activation. This possible MoA is generally considered to be qualitatively not plausible for humans (Elcombe *et al* 2014). The studies assess CYP enzyme induction, cytotoxicity and replicative DNA synthesis in isolated rat and human hepatocytes.

The studies are summarised below. Their relevance to the assessment of fluxapyroxad carcinogenicity and other potential mechanisms noted above is discussed in Section 10.9.4.1 .

10.9.3.1 *In vitro* studies with rat microsomes

Table 25: *In vitro* studies with rat microsomes

Type of study/data Method	Test substance,	Relevant information about the study (as applicable) Observations	Reference
Enzyme inhibition Study to investigate the potential inhibition of CYP2B and CYP3A activity by Fluxapyroxad Mechanistic study (No guideline available) non-GLP Liver microsomes from Phenobarbital (PB) and 5-Pregnen-3β-ol-20-one 16α-carbonitrile (PCN) induced SD rats were prepared and the activity of CYP2B and CYP3A was determined using the PROD and BQ assay PROD: Pentoxyreso-rufin-O-depentylase; BQ: Benzyloxyquinoline-O-debenzylation	Fluxapyroxad 99.2% 0.1 to 100 µM	Fluxapyroxad inhibited the CYP2B enzyme activity in liver microsomes from PB induced rats with an IC_{50} of 0.87 µM. No substantial inhibition of CYP3A from PCN induced liver microsomes was observed. At a Fluxapyroxad concentration of 100 µM the BQ assay revealed an activity of 69% of the control, i.e. the IC_{50} is > 100 µM.	Anonymous (2016)

In a limited study to investigate the potential of fluxapyroxad to inhibit rat liver microsomes, inhibition of CYP2B was observed but not of 3A, at concentrations of up to 100 µM fluxapyroxad

(Anonymous (2016)). An IC_{50} of 0.87 µM was determined for CYP 2B inhibition, indicating fluxapyroxad is a relatively potent inhibitor of CYP 2B activity, but does not inhibit CYP 3A.

10.9.3.2 *In vitro* studies with rat hepatocytes

Three studies are available, two conducted using hepatocytes from Sprague-Dawley rats and one using the Wistar rat strain (the strain of rat used in the 2-year carcinogenicity study).

Study 1

A study to investigate enzyme activity and DNA-synthesis specific to CAR activation, cytotoxicity and cell proliferation (Elcombe B. (2016a)). Primary hepatocytes were pooled from 2 wild type (WT) and 2 CAR knock out (CAR KO) Sprague-Dawley rats. Treatment with test substance (fluxapyroxad; 99.2% pure at 10, 30, 100 and 300 µM), vehicle (DMSO) or control substances (phenobarbital (PB); 100 and 1000 µM or Epidermal Growth Factor (EGF); 25 ng/ml) was for 3 days. At the end of treatment, cells were either methanol fixed (determination of replicative DNA synthesis via BrdU incorporation and immune-staining) or scraped off and sonicated prior to determination of ATP levels (measure for cytotoxicity) or biochemical assays to determine CYP2b and CYP3A activity (PROD, BROD, BQ assays).

Table 26: Summary of i*n vitro* study with hepatocytes from wild type and CAR knock-out Sprague-Dawley rats to investigate enzyme activity, cytotoxicity and cell proliferation

Type of data	Relevant information about the study (as applicable) (values are compared to controls)	Reference
Cytotoxicity assay	**Fluxapyroxad** ↓ ATP at 300 µM (1.3% / 0.8% (WT/CAR KO),	Elcombe B. (2016a) BASF DocID 2016/1091503
Enzyme Induction PROD (CYP2B): Pentoxyreso-rufin-O-depentylase; BROD (CYP2B/CYP3A): Benzyloxyresorufin-O-debenzyl-ase; BQ (CYP3A): Benzyloxyquinoline-O-debenzylation	**WT hepatocytes**: (maximum activity of) **Fluxapyroxad** ↑ PROD (CYP2B): 7.2x at 10 µM, decreased to 1.6x at 300 µM ↑ BROD (CYP2B&3A): 13x at 30 µM, decreased to 4.1x at 300 µM ↑BQ (CYP3A): 8.1x at 100 µM, decreased to 3.3x at 300 µM; **PB** ↑PROD: 5.7x /8.2x at 100/1000 µM ↑BROD: 9.2x/14.8x at 100/1000 µM ↑BQ: 2.5x at 1000 µM **CAR KO hepatocytes**: (maximum activity of) **Fluxapyroxad** ↑ PROD (CYP2B): 1.7x to 2x at 10 to 300 µM ↑ BROD (CYP2B&3A): 3.5x at 30 µM, decreasing to 1.5x at 300 µM ↑BQ (CYP3A): 6.9x at 100 µM, decreasing to 5.5x at 300 µM **PB** ↑PROD 1.7x at 1000 µM ↑BROD: 1.2x at 1000µM ↑ BQ (CYP3A): 2.5x at 1000 µM	
Replicative DNA Synthesis	**Labelling Index** WT hepatocytes: **Fluxapyroxad**: ↑ 1.5x at 10 µM, decreasing to 0.86x at 300 µM; **PB**: ↑ 1.8x at 100 and 1000 µM; **EGF:** ↑ 4.1x CAR KO hepatocytes: **Fluxapyroxad:** ↓ 0.34x at 100 µM, (cytotox at 300 µM) **PB:** no change **EGF:** ↑ 4.3x	

The response of wild type (WT) and CAR knock out (CAR KO) primary Sprague-Dawley (SD) hepatocytes to fluxapyroxad treatment was investigated (Elcombe B. (2016a). Phenobarbital (PB) served as a positive control. Decreased CYP2B enzyme activity as measured by PROD and BROD at >10 µM, respectively >30 µM. This was due to an inhibition of CYP2B activity by Fluxapyroxad.

The ATP assay indicated that fluxapyroxad may be slightly cytotoxic but only at the highest dose tested (300 µM),

A proliferative response was noted in WT hepatocytes whereas there was no induction of replicative DNA synthesis noted in CAR KO hepatocytes following treatment with fluxapyroxad and phenobarbital. The ability of both the WT and CAR KO hepatocytes to proliferate was demonstrated by treatment with EGF which resulted in an ~4x increase in replicative DNA synthesis.

Study 2

A study to investigate enzyme activity, mRNA and DNA-synthesis specific to CAR activation, cytotoxicity and cell proliferation (Elcombe B. (2016c)). Primary hepatocytes were pooled from wild type (WT) and CAR knock out (CAR KO) Sprague-Dawley rats. Prior to treatment, hepatocytes were cultured for 4 hours in order to adhere. Treatment with test substance (fluxapyroxad; 99.2% pure at 1, 2, 4, 8 and16 µM) , vehicle (DMSO) or control substances (Phenobarbital sodium salt (PB) 500 µM or Epidermal Growth Factor (EGF) 25 ng/ml) was for 3 days. At the end of treatment cells were either methanol fixed (determination of replicative DNA synthesis via BrdU incorporation and immune-staining) or scraped off and sonicated prior to determination of ATP levels (measure for cytotoxicity) or biochemical assays to determine CYP1A, CYP2B, CYP3A and CYP4A activity (EROD, PROD, BROD, BQ, LAH assays). PPARα induced peroxisome proliferation was measured by acyl CoA oxidation using palmitoyl-CoA.

Table 27: Summary of i*n vitro* study with hepatocytes from wild type and CAR knock-out Sprague-Dawley rats to investigate enzyme activity, cytotoxicity and cell proliferation

Type of data	**Relevant information about the study (as applicable) (values are compared to controls)**	**Reference**
Cytotoxicity assay	No biologically significant decrease of ATP levels observed with fluxapyroxad or PB	Elcombe B. (2016c) BASF DocID 2015/1243689
Enzyme Induction Liver microsomes analysed for specified enzyme activity using standard assays: EROD (CYP1A1) Ethoxyresofufin-O-deethylase PROD (CYP2B): Pentoxyresorufin-O-depentylase; BROD (CYP2B/CYP3A): Benzyloxyresorufin-O-debenzyl-ase; BQ (CYP3A): Benzyloxyquinoline-O-debenzylation PCoA LAH (CYP 3A4) Lauric acid hydrolylase	**<u>WT hepatocytes</u>: (m**aximum activity of**)** **Fluxapyroxad** ↑ EROD: 3.8x at 16 µM ↑ PROD: 3.8x at 16 µM ↑ BROD: 10.2x at 16 µM ↑ BQ: 4.0x at 16 µM ↓ PCoA: 0.47x at 16 µM LAH: No Change **PB** ↑ EROD: 2.8x ↑ PROD: 4.5x ↑ BROD: 10.6x ↑ BQ: 2.8x ↑ LAH: 1.7x ↑ PCoA: 1.3x	

Type of data	Relevant information about the study (as applicable) (values are compared to controls)	Reference
	CAR KO hepatocytes: (maximum activity of) **Fluxapyroxad** ↑ EROD: 5.8x at 16 µM ↑ PROD: 1.4x at16 µM ↑ BROD: 3.5x at 16 µM ↑ BQ: 5.8x ↑ PCoA: 2.4x at 8 µM LAH: No Change **PB** ↑ EROD: 3.1x ↑ PROD: 1.3x ↑ BROD: 2.1x ↑ BQ: 1.7x ↑LAH: 1.3x ↑PCoA: 1.3x	
Messenger RNA Analysis Taqman® analysis was performed for CYP1A1, CYP1A2, CYP2B1, CYP2B2, CYP3A1, CYP4A1, Acox1 mRNA. Rat specific β-actin was used as the internal standard.	**WT hepatocytes (**maximum activity of) **Fluxapyroxad** ↑ CYP1A1 mRNA: 2.0x at 16 µM ↑CYP1A2 mRNA: 2.5x at 16 µM ↑ CYP2B1 mRNA: 212x at 16µM ↑ CYP2B2 mRNA: 296x at 8 µM ↑ CYP3A1 mRNA: 49x at 16 µM ↓ CYP4A1 mRNA: 0.5x at 2 µM, ↑ Acox1 mRNA: 1.6x at 16 µM **PB** ↑ CYP1A1 mRNA: 2.5x ↑ CYP1A2 mRNA: 1.9x ↑ CYP2B1 mRNA: 396x ↑ CYP2B2 mRNA: 668x ↑ CYP3A1 mRNA: 32x ↓ CYP4A1 mRNA:2.3x ↑ Acox1 mRNA: 1.5x **CAR KO hepatocytes (**maximum activity of)) **Fluxapyroxad** ↑ CYP1A1 mRNA: 1.7x at 8 µM ↑ CYP1A2 mRNA: 1.9x ↑ CYP2B1 mRNA: 6.3x at 16µM ↑ CYP2B2 mRNA: 2.1x at 8 µM ↑ CYP3A1 mRNA: 53x at 16 µM ↓ CYP4A1 mRNA: 0.2x at 16 µM ↔ Acox1 mRNA: No Change	

Type of data	Relevant information about the study (as applicable) (values are compared to controls)	Reference
	PB ↑ CYP1A1 mRNA: 2.5x ↑ CYP1A2 mRNA: 1.2x ↑ CYP2B1 mRNA: 1.5x ↑ CYP2B2 mRNA: 1.8x ↑ CYP3A1 mRNA: 8.5x ↓ CYP4A1 mRNA: 0.9x ↔ Acox1 mRNA: No Change	
Replicative DNA Synthesis	**Labelling Index** WT hepatocytes: **Fluxapyroxad:** ↑ 2.0x at 8 µM and 1.9x at 16 µM **PB:** ↑ 1.4x; **EGF:** ↑**4.9x** CAR KO hepatocytes: **Fluxapyroxad** : No Change **PB:** No change **EGF:** ↑ 4.2x	

Incubation of SD WT hepatocytes with Fluxapyroxad resulted in a marked increase of CAR mediated CYP2B1 (max. 212x), CYP2B2 (296x) and CYP3A1 mRNA (max. 49x) (Elcombe B. (2016c)). In sharp contrast CYP2B1 and CYP2B2 mRNA increased only marginally by max. 6.3x and 2.1x in CAR KO hepatocytes, confirming that CPY2B mRNA induction is CAR mediated. As to be expected, the PXR mediated increase of CYP3A1 mRNA was essentially comparable in WT and CAR KO hepatocytes (49x vs. 53x), as both types of hepatocytes had intact PXR receptors.

Parallel to increased mRNA levels, CYP2B dependent hepatic PROD activity was dose dependently increased up to 3.8xd in SD WT hepatocytes, while PROD was only slightly increased (max. 1.4x) in CAR KO hepatocytes. BROD was increased up to 10.2x in WT hepatocytes, whereas in CAR KO hepatocytes the increase was up to 3.5x. The relatively high increase in CAR KO hepatocytes is explained by the fact that benzoyloxyresorufin is not only debenzylated by CYP2B but also by CYP3A, which is not decreased in its activity because the PXR receptor is intact in CAR KO hepatocytes. Accordingly, the CYP3A mediated benzyloxyquinoline-O-debenzylation (BQ) was 4.0x and 5.8x increased in WT and CAR KO hepatocytes.

Induction of AhR mediated CYP1A1 and CYP1A2 mRNA by Fluxapyroxad was marginal in WT and CAR KO hepatocytes (up to 2.5x and 1.9x, respectively). TDCC as prototypical AhR receptor agonists led to an up to 54x increase of mRNA (Budinsky et al. 2010[1]). At the level of CYP1A catalysed enzyme activity , a slight increase of EROD both in WT (3.8x) and CAR KO hepatocytes (5.8x) was noted. This increase is small compared to the response of prototypical AhR receptor agonists like TCDD (18x) or the respective dibenzofuran TCDF (35x) (Budinsky et al. 2010). Based on these results there is no evidence for a specific AhR activation by Fluxapyroxad.

[1] Budinsky R.A., LeCluyse E.L., Ferguson S.S, Rowlands J.C., Simon T.(2010). Human and rat primary hepatocyte CYP1A1 and 1A2 induction with 2,3,7,8-tetrachlorodibenzo-p-dioxin, 2,3,7,8-tetrachlorodibenzofuran, and 2,3,4,7,8-pentachlorodibenzofuran. Toxicol. Sci, 118(1), 224–35

PPARα mediated induction of CYP4A1 mRNA level in WT and CAR KO hepatocytes was actually decreased by Fluxapyroxad. Likewise, the second PPARα dependent mRNA investigated, i.e. Acox 1 (Peroxisomal acyl-Coenzyme A oxidase 1), was marginally induced in WT hepatocytes (max. 1.6x) and unaltered in CAR KO hepatocytes. This is paralleled by a minimal, dose dependent increase of CYP4A catalysed 12-hydroxylation of lauric acid (LAH). Likewise, there was no evidence for PPARα induced lipid peroxidation as investigated by palmitoyl-CoA oxidation (PCoA), which was lower in WT hepatocytes (min. 0.47x), but slightly increased in CAR KO hepatocytes (max. 2.4x). As for AhR there is no evidence for a specific PPARα activation by Fluxapyroxad.

Hepatocellular proliferation was determined in WT and CAR KO hepatocytes by the incorporation of BrdU followed by immuno-staining. After 3 days of Fluxapyroxad treatment a dose-dependent increase of replicative DNA synthesis was noted, plateauing at about a 2x increase at concentration of 4 to 16 µM. In sharp contrast, no induction of replicative DNA synthesis was observed in CAR KO hepatocytes after Fluxapyroxad treatment, indicating that the CAR nuclear receptor is essential for the induction of cellular proliferation. The principal ability of CAR KO hepatocytes to proliferate was demonstrated by treatment with epidermal growth factor (EGF), which resulted in a 4.2x increase of replicative DNA synthesis.

Study 3

A study to investigate enzyme activity, mRNA and DNA-synthesis specific to CAR activation, cytotoxicity and cell proliferation (Elcombe B. (2016d). This study employed the same protocol as the previous study (study 2), with the exception that the hepatocytes were obtained from Wistar rats (the strain of rat used in the 2-year carcinogenicity study) and the doses of fluxapyroxad employed were 1, 3, 10, 30 and 100 µM (99.2% pure)

Table 28: Summary of *in vitro* study with hepatocytes from Wistar rats to investigate enzyme activity, cytotoxicity and cell proliferation

Type of data	Relevant information about the study (as applicable) (values are compared to controls)	Reference
Cytotoxicity assay	**Fluxapyroxad** No biologically significant decrease of ATP levels observed. **PB** Not affected	Elcombe B. (2016d) BASF DocID 2016/1084305
Enzyme Induction Liver microsomes analysed for specified enzyme activity using standard assays: EROD (CYP1A1) Ethoxyresofufin-O-deethylase PROD (CYP2B): Pentoxyresorufin-O-depentylase; BROD (CYP2B/CYP3A): Benzyloxyresorufin-O-debenzyl-ase; BQ (CYP3A): Benzyloxyquinoline-O-debenzylation PCoA LAH (CYP 3A4) Lauric acid hydrolylase	Maximum activity of**:** **Fluxapyroxad** ↑ EROD: 6.5x at 100 µM ↑ PROD: 13.3x at 10 µM ↑ BROD: 11.9x at 10 µM ↑ BQ: 8.6 x at 3016 µM ↓ PCoA: 0.65x at 30 µM LAH: 2.7x at 30 µM **PB** ↑ EROD: 2.7x ↑ PROD: 11.7x ↑ BROD: 9.5x ↑ BQ: 2.5x ↑ LAH: 1.7x PCoA: no change	

Type of data	Relevant information about the study (as applicable) (values are compared to controls)	Reference
Messenger RNA Analysis Taqman® analysis was performed for CYP1A1, CYP1A2, CYP2B1, CYP2B2, CYP3A1, CYP4A1, Acox1 mRNA. Rat specific β-actin was used as the internal standard.	Maximum activity of**:** **Fluxapyroxad** ↑ CYP1A1 mRNA: 2.5x at 100 µM ↑ CYP1A2 mRNA: 4.6x at 100 µM ↑ CYP2B1 mRNA: 46x at 10µM ↑ CYP2B2 mRNA: 18.6x at 10 µM ↑ CYP3A1 mRNA: plateau 70x at ≥ 30 µM ↓ CYP4A1 mRNA: 0.25x at 30 µM ↑ Acox1 mRNA: No change **PB** ↑ CYP1A1 mRNA: 1.8x ↑ CYP1A2 mRNA: 1.9x ↑ CYP2B1 mRNA: 100x ↑ CYP2B2 mRNA: 56x ↑ CYP3A1 mRNA: 23x ↓ CYP4A1 mRNA: No change ↑ Acox1 mRNA: No change	
Replicative DNA Synthesis	**Labelling Index** **Fluxapyroxad:** ↑ Peak at 10 µM (2.6x), decreasing to 1.5x at 100 µM **PB:** ↑ 1.4x **EGF:** ↑5.0x	

In a study using hepatocytes from Wistar rats (Elcombe B. (2016d)) the same effects on mRNA induction, enzyme activities and hepatocellular proliferation were observed as in WT SD hepatocytes, thus indicating that – in the absence of a respective CAR KO model in Wistar rats - the mechanistic information obtained from the study in Sprague-Dawley WT and CAR KO hepatocytes is applicable to the Wistar rat too. Numerical differences in the extent of cellular responses of Wistar and SD hepatocytes were to a considerable extent due to the different concentrations used in both the studies (1 to 100 µM in Wistar vs. 1 to 16 µM in SD WT and CAR KO hepatocytes).

10.9.3.3 *In vitro* studies with Human hepatocytes

A study to investigate the effects of fluxapyroxad on enzyme induction, cytotoxicity and cell proliferation was conducted in human hepatocytes. This study employed the same principal protocol as the previous studies (study 2 and 3).

Cryopreserved primary human hepatocytes from two male donors were cultured with 1, 3, 10, 30 and 100 µM fluxapyroxad (99.2%), Additional cultures were exposed to phenobarbital sodium salt - PB (500 µM) for comparative purposes. Epidermal growth factor – EGF (25 ng/ml) was employed as a positive control to demonstrate the inherent capacity of these cells to undertake replicative DNA synthesis.

Donor 1 was a Caucasian male, 51 years old, with no reported medication. Donor 2, a 39 year old Caucasian male, was reported to be suffering from multiple sclerosis with multiple medical drug treatments and positive urine test for drugs including THC, opiates and cocaine.

Table 29: Summary of *in vitro* study with human hepatocytes to investigate enzyme activity, cytotoxicity and cell proliferation

Type of data	Relevant information about the study (as applicable) (values are compared to controls)	Reference
Cytotoxicity assay	**Fluxapyroxad** Donor 1 ↓ ATP by 59% at 100 µM; Donor 2 ↓ ATP by 77% at 100 µM	Elcombe B. (2016e) BASF DocID 2015/1184808
Enzyme Induction Liver microsomes analysed for specified enzyme activity using standard assays: PROD (CYP2B): Pentoxyresorufin-O-depentylase; BROD (CYP2B/CYP3A): Benzyloxyresorufin-O-debenzyl-ase; BQ (CYP3A): Benzyloxyquinoline-O-debenzylation	**Donor 1**(maximum activity of) **Fluxapyroxad** ↑ PROD: 1.8x at 30 µM ↑ BROD: 2.9x at 30 µM ↑ BQ: 2.6x at 10 µM **PB** ↑ PROD: 2.4x ↑ BROD: 5.5x ↑ BQ: 5.3x **Donor 2** (maximum activity of) **Fluxapyroxad** ↑ PROD: 0.6 to 0.7x at 1 to 30 µM ↑ BROD: 1.8x at 100 µM ↑ BQ: 2.5x at 30 µM **PB** ↑ PROD: 1.3x ↑ BROD: 3.1x ↑ BQ: 3.9x	
Messenger RNA Analysis	**Donor 1** **Fluxapyroxad** ↑ CYP2B6 mRNA: 4.4x at 30 µM ↑ CYP3A4 mRNA: 3.7x at 10 µM **PB** ↑ CYP2B6 mRNA: 7.4x ↑ CYP3A4 mRNA: 7.5x **Donor 2** **Fluxapyroxad** ↑ CYP2B6 mRNA: 3.3x at 30 µM ↑ CYP3A4 mRNA: 1.8x at 30 µM **PB** ↑ CYP2B6 mRNA: 8.2x ↑ CYP3A4 mRNA: 5.9x	
Replicative DNA Synthesis	**Labelling Index** Donor 1 **Fluxapyroxad:** No change **PB:** ↑1.2x **EGF:** ↑13.5x	

Type of data	Relevant information about the study (as applicable) (values are compared to controls)	Reference
	Donor 2: **Fluxapyroxad** : No change **PB:** ↑1.3x **EGF:** ↑ 7.2x	

The induction of CYP2B6 and CYP3A4 mRNA, PROD, BROD and BQ enzyme activity and hepatocellular proliferation was investigated in cryopreserved primary hepatocytes from two male Caucasian donors (Elcombe B (2016e)). For Donor 1, except for a sporadic use of alcohol, no exposure to tobacco, drugs or medication was reported. Donor 2, suffering from multiple sclerosis, was a smoker with indication of alcohol and drug abuse and intensive medication. This may have resulted in the relative high baseline level of CYP3A4 mRNA in this Donor. Despite numerical differences between the hepatocytes of the two donors, both reacted within the range observed in human hepatocytes in 19 studies performed between 2010 and 2015 with regard to basal CYP2B6 and CYP3A4 activity as well as for PB induced increases of enzyme activity. Fluxapyroxad treatment of human hepatocytes resulted in a maximum 4.4x and 3.3x increase of CYP2B6 mRNA in donors 1 and 2, respectively. The induction of CYP3A4 mRNA was up to 3.7x and 1.8-x, respectively.

In hepatocytes from Donor 1 Cyp2B6 activity as determined by PROD and BROD peaked at 30 µM with 1.8x and 2.9x increases, respectively. Cyp3A4 (BQ) activity peaked at 10 µM with a 2.6x increase. The decline of enzyme activities observed at 100 µM may be the result of cytotoxicity (decrease of ATP levels by approx. 41%) and/or CYP P_{450} inhibition. In Donor 2 the response was less pronounced (PROD 1.2x, BROD 1.8x, BQ 2.5x). Likewise the increase of enzyme activity after PB treatment was lower (PROD 1.3x, BROD 3.1x, BQ 3.9x) than in hepatocytes of Donor 1 (PROD 2.4x, BROD 5.5x, BQ 5.3x). Nonetheless, the enzyme induction pattern in hepatocytes of both donors was comparable.

Replicative DNA synthesis was not increased in either donor. This is in contrast to the Fluxapyroxad induced hepatocellular proliferation in wild type hepatocytes from Sprague-Dawley and Wistar rats. Treatment of human hepatocytes with EGF resulted in a 13.5x and 7.2x increase of replicative DNA synthesis indicating that the hepatocytes were able to proliferate.

10.9.3.4 *In vivo* hepatocellular proliferation

Three studies are available, specifically designed to investigate the potential of fluxapyroxad to induce a proliferative response in rat hepatocytes, as measured by S-phase BRDU labelling.

Table 30: Summary of *in vivo* studies investigating the proliferative response in rat hepatocytes

Type of study/data Method	Test substance,	Relevant information about the study (as applicable) Observations	Reference
Proliferation GLP Dietary administration Wistar rat (Crl:WI Han) 10/group/sex; sacrifice after 7, 28, and 91 days of treatment; Recovery group: 28-day treatment, 28.day recovery. Age of all main group animals was the same at sacrifice as treatment of the 7 and 28-day groups started 7 or 28-days prior to sacrifice. BrdU: 5-bromo-2´-deoxyuridine	Fluxapyroxad 99.2% 0, 250, 1500, 3000 ppm Recovery group 0, 3000 ppm males: 13, 80 and 183 (91 day admin.), 12, 79, 122/131# (28-day/ # 28-day & 28-day recovery), 12, 61, 104 (7 day admin.) – all values mg/kg bw/day; females: 17, 106 and 190 (91 day admin.), 15, 87, 173/1172# (28-day/ # 28-day & 28-day recovery), 18 79, 137 (7 day admin.) – all values mg/kg bw/day;	**<u>91 day treatment:</u>** **General toxicity** ↓ bw 4%/7% (m/f), ↓ bwg 10%/26% (m/f*) at 3000ppm ↑ Liver weight abs/rel wt up to 141%/ 147% of control at ≥1500 ppm (m/f)*; ↑ rel thyroid weight at ≥1500 ppm (f*) up to 128% Liver enlarged ≥ 1500 ppm (m/f); teeth: discoloration ≥ 1500 ppm hepatocellular hypertrophy, increased incidence and severity at ≥ 250 ppm (m/f) **Proliferation** S-phase response – ↑ males 3000 ppm, zone 3, 2.8x; females ≥ 250 ppm, zone 3, up to 7.1x **<u>28 day treatment:</u>** **General toxicity** ↓ bw 6%/4% (m/f), ↓ bwg 20%/18% (m/f) at 3000 ppm ↑ Liver weight abs/rel at ≥1500 ppm (m/f)* up to 142%/ 145%; almost fully reversible within 28-day (rel. liver weight in females slightly increased (111% of ctrl)) Liver enlarged ≥ 1500 ppm (m) 3000 ppm (f); almost fully reversible within 28-days (2/10 compared 10/10 at the end of the treatment period) Histopathology – hepatocellular hypertrophy, increased incidence and severity at ≥ 250 ppm (m/f); fully reversible within 28-days **Proliferation** S-phase response – ↑ females ≥ 250 ppm, zone 3, up to 13.7x; 3000 ppm, zone 2, 1.6x; Labelling index in 3000 ppm recovery group animals was decreased in both sexes **<u>7 day treatment:</u>** **General toxicity** ↓ bw 2%/5% (m/f*); ↓ bwg 3%/19% (m/f*) at 3000ppm ↑ Liver weight abs/rel wt ≥1500 ppm (m/f)* up to 128%/130%	Anonymous (2010b)

Type of study/data Method	Test substance,	Relevant information about the study (as applicable) Observations	Reference
		Liver enlarged 3000 ppm (m) Histopathology – hepatocellular hypertrophy, increased incidence and severity at ≥ 250 ppm (m/f) **Proliferation** S-phase response – ↑ males ≥ 1500 ppm, zone 1/2/3, up to 4.4x/8.3x/21.2x; ↑ females > 250 ppm, zone 1/2/3, up to 5.6x/16.2x/25.6x	
Proliferation S-phase response study GLP Dietary administration Wistar rat (Crl:WI Han) 10/group/sex; sacrifice after 7, 28, and 91 days of treatment. Age of all main group animals was the same at sacrifice as treatment of the 7 and 28-day groups started 7 or 28-days prior to sacrifice. BrdU: 5-bromo-2´-deoxyuridine	Fluxapyroxad 99.2% 0, 50 ppm 7-days: 2.5/2.9 mg/kg bw/day (m/f) 28-days: 2.5/3.1 mg/kg bw/day (m/f) 91-days: 2.8/3.2 mg/kg bw/day (m/f)	**General toxicity** No mortality and no signs of toxicity were observed. No effects on body weight or organ weight and no macroscopic or microscopic findings were observed. **Proliferation** S-phase response: No treatment-related effects. A statistically, but not biologically significant increase of the labelling index in females after 28-day in Zones 1 (↑ 1.4x) and 2 (↑ 1.9x) was observed.	Anonymous (2010c)
Proliferation (up to 14-days) S-phase response study GLP Dietary administration Wistar rat (Crl:WI Han) 10/group/sex; sacrifice after 1, 3, 7, and 14 days of treatment. Age of all main group animals was the same at sacrifice as treatment of the 1, 3 and 7 groups started 1, 3 and 7 days prior to sacrifice, i.e. these animals had a treatment-free period prior to administration. BrdU: 5-bromo-2´-deoxyuridine	Fluxapyroxad 99.2% 0, 50, 250, 1500, 3000 ppm males: 4.0, 17, 106 and 201 (14 day admin.), 3.3, 16, 100 and 183 (7-day admin.), 3.0, 16, 93 and 176 (3 day admin.), 3.0, 15, 86 and 150 (1 day admin.) – all values mg/kg bw/day; females: 3.5, 20, 104 and 214 (14 day admin.), 3.5, 17, 92 and 195 (7 day admin.), 3.2, 15, 82, 186 (3 day admin.), 3.6, 17, 91 and 146 (1 day admin.) – all values	No mortality and no signs of toxicity were observed. No biologically significant effects on body weight. **<u>14 day treatment:</u>** **General toxicity** ↑ Liver weight abs/rel up to 144%/ 148% at ≥ 250 ppm (m*) and ≥ 1500 ppm (f*); ↑ abs/rel thyroid wt at ≥ 50 ppm (m*)up to 139%/137%# Liver enlarged ≥ 1500 ppm (m/f) Histopathology – hepatocellular hypertrophy, increased incidence and severity at ≥ 250 ppm (m) and ≥ 1500 ppm (f); **Proliferation** S-phase response – ↑ males ≥ 1500 ppm, zone 3, up to 2.0x; females ≥ 250 ppm, zone 3, up to 10.9x, ≥ 1500 ppm, zone 2, up to 3.7x **<u>7 day treatment:</u>** **General toxicity** ↑ abs/rel Liver weight at ≥ 1500 ppm (m/f)* up to 134%/ 137%; ↑ abs/rel thyroid weight at ≥ 50 ppm (m*) up to 128%/131%# Liver enlarged ≥ 1500 ppm (m/f) Histopathology – hepatocellular hypertrophy, increased incidence and severity at ≥ 250 ppm (m), 3000 ppm (f)	Anonymous (2010d)

Type of study/data Method	Test substance,	Relevant information about the study (as applicable) Observations	Reference
	mg/kg bw/day;	**Proliferation** S-phase response – ↑ males ≥ 1500 ppm, zones 1/2/3, up to 7.2x/8.0x/14.8x; ↑ females ≥ 1500 ppm, zones 1/23, up to 6.5x/17.3x/15.5x **3 day treatment:** ↑ abs/rel liver weight ≥ 1500ppm (m/f)* up to 128%/131%; ↑ abs/rel thyroid weight at ≥ 50 ppm (m*) up to 128%/128%# Liver enlarged ≥ 1500 ppm (m/f)) Histopathology – hepatocellular hypertrophy, increased incidence and severity at 3000 ppm (f) **Proliferation** S-phase response – ↑ males ≥ 1500 ppm, zone 1/2/3, up to 5.3x/7,3x/10,5x; ↑ females > 250 ppm, zone 1/2/3, up to 4.4x/10.3x/10.1x **1 day treatment:** No general toxicity or adverse effects on proliferation were observed at this time point. # As thyroid weight changes displayed no dose-response relationship the study director considered these to be unrelated to treatment.	

* Indicates statistically significant, $p \leq 0.01$

In a set of three *in-vivo* S-Phase response studies (Anonymous (2010b, 2010c, 2010d)) hepatocellular cell proliferation was determined via BrdU incorporation after 1, 3, 7, 14, 28 and 91 days of administration at the dose levels employed in the rat long-term study (see above). The reversibility of the effects was investigated at the high dose after 28-days of treatment in a 28-day recovery group. As early as 3 days after commencement of treatment a dose dependent increase of S-Phase response was noted, which peaked after 7 days and declined afterwards. No increased S-Phase response was noted in males at days 28 and 91, while a slight S-Phase response was still noted in females after 91-days of treatment. Like S-Phase response, a dose-dependent increase of liver weights and of the incidence and severity of hepatocellular hypertrophy was noted from treatment day 3 onwards. In contrast to S-Phase response, liver weights and hepatocellular hypertrophy stayed increased throughout treatment as increased liver weights and hepatocellular hypertrophy were observed throughout the long-term study in rats.

10.9.3.5 In vivo enzyme induction study

In a 14-day enzyme induction study in Wistar rats employing the dose levels of the long-term rat study (0, 50, 250, 1500 and 3000 ppm) and including a 28-day recovery period at the high dose (Anonymous (2009f and 2010a) see table 31 in section 10.9.3.6 below), a dose-dependent increase of total cytochrome P_{450} 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. The increase of EROD representative for CYP1A enzyme activity was marginal compared to the increased observed with AhR agonists. The higher induction of PROD and BROD (representative for CYP2B) enzyme activity in females was due to considerably lower baseline levels – the absolute PROD and BROD enzyme activities in treated groups were lower than in males. A dose-

dependent increase in liver weights accompanied by an increase in the incidence and severity of centrilobular hepatocyte hypertrophy was noted. The induction of total CYP content and CYP enzyme activity was reversible within the 28-day recovery period. This holds true for liver weights and hepatocellular hypertrophy.

Mechanistic studies relevant for findings in the thyroid

There are a number of recognised MoA for perturbation of the rat HPT axis; inhibition of Type I or II DI, interference with production of T4 and T3 in the thyroid via inhibition of thyroid peroxidase (TPO), interference with iodide uptake into the thyroid via the sodium/iodide symporter (NaIS) and induction of phase II liver glucuronyl transferases involved in the of T3 and T4.

Several nonstandard studies have been conducted to investigate the MoA for induction of thyroid follicular tumours. 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) and early changes in thyroid hormone levels. All these studies were conducted *in vivo*. No other potential MoA were investigated.

10.9.3.6 In vivo enzyme induction

Two non-standard studies are available, which investigated the potential of fluxapyroxad to induce some hepatic cytochromes P450 and perturb the HPT axis, both were conducted in rats.

Study 1

A 14-day, GLP, Phase I and Phase II enzyme induction study was conducted in Wistar rats (10/sex/group). Fluxapyroxad (99.2%) was administered in the diet at dose levels of 0, 250, 1500, 3000 ppm (corresponding to 16, 96 and 192 mg/kg bw/day in males and 19, 126, 234 mg/kg bw/day in females. The study included a 28-day recovery period at 0 and 3000 ppm.

Table 31: Summary of *in vivo* study to investigate enzyme induction in Wistar rats

Type of data	Relevant information about the study (as applicable)	Reference
General toxicity	No mortality and no signs of toxicity ↓ bw gain 6%/21% (m/f*) in main group; no significant effects in recovery animals ↑ abs Liver wt (m/f)* at ≥ 250 ppm: up to 52% in males and up to 44% in females ; reversible in recovery group except for 3000 ppm males (↑12% abs/↑14% rel wt) Histopathology: Liver: ↑ centrilobular hepatocellular hypertrophy at ≥ 250 ppm, males: incidence 1, 6, 9, 10 and females 0, 2, 10, 10; reversible within recovery period. Thyroid: ↑ follicular hypertrophy/hyperplasia: males: 0, 2, 4, 5 - females 0, 0, 0, 4; ↑ altered colloid: males 0, 1, 1, 2; all findings reversible within recovery period	Anonymous (2009f)

Type of data	Relevant information about the study (as applicable)	Reference
Enzyme Induction Liver microsomes assayed for specified enzyme activities , CYP1A and CYP2B enzyme activity (EROD, PROD, BROD), Glucuronyl transferase activity (T_4-UDP GT, MUF-GT, HOBI-GT) EROD: Ethoxyresorufin-O-deethylase; PROD: Pentoxyreso-rufin-O-dependentylase; BROD: Benzyloxyresorufin-O-debenzyl-ase; T_4-UDP-GT: T_4-specific UDP-glucuronosyltransferase; MUF-GT: 4-Methylumbeliferone-glucuronyl-transferase; HOBI-GT: 4-Hydroxy-biphenyl-glucuronyltransferase	EROD – males: 1.83x**, 3.10x**, 3.12x**; females: 1.66x**, 2.74x**, 2.75x**; reversible within recovery period PROD – males: 3.93x**, 17.6x**, 20.4x**; females: 23.8x**, 106x**, 125x**; reversible within recovery period BROD – males: 3.26x**8.99x**, 9.94x**; females: 32.8x**, 116x**, 127x; recovery: reversible in males, females: 4.7x MUF-GT – males: 1.62x**, 3.42x**, 4.69x**; females: 1.55x**, 3.06x**, 3.70x**; recovery: males: 1.48x**; females reversible HOBI-GT – males: 1.90x**, 3.81x*, 4.67x**; females: 1.84x**; 2.94x**, 3.14x**; reversible within recovery period T_4-UDP-GT: males: 1.1x, 1.52x**, 1.58x**; females: 1.81x, 2.38x**, 2.68x**; reversible within recovery period	
Cytochrome P450 content	**Total CypP450** - males: 1.26x**, 1.99x**, 2.11x**; females: 1.32x*, 1.89x**, 1.83x** at 250, 1500 and 3000 ppm, respectively; reversible within recovery period	
Thyroid Hormones T3, T4, TSH levels in serum	↑ TSH (males*) at 3000 ppm in main group, n.s. increase at 1500 ppm; fully reversible in recovery group No changes in T3 or T4	

* Indicates statistically significant, $p \leq 0.01$
** Indicates statistically significant, $p \leq 0.01$ (Wilcoxon test)

Study 2

A 14-day, GLP, Phase I and Phase II enzyme induction study was conducted in Wistar rats (10/sex/group). Fluxapyroxad (99.2%) was administered in the diet at dose levels of 0, and 50ppm (corresponding to o and 3.0 mg/kg bw/day in males and 0 and 3.8 mg/kg bw/day in females. The study included a 28-day recovery period at 0 and 3000 ppm.

Table 32: Summary of *in vivo* study to investigate enzyme induction in Wistar rats

Type of data	Relevant information about the study (as applicable)	Reference
Study 2		
General Toxicity	No mortality, clinical signs or effects on body weight development. No statistically significant effects on liver or thyroid weights	Anonymous (2010e)
Enzyme Induction CYP1A and CYP2B enzyme activity (EROD, PROD, BROD), Glucuronyl transferase activity (T_4-UDP GT, MUF-GT, HOBI-GT) EROD: Ethoxyresorufin-O-deethylase; PROD: Pentoxyreso-rufin-O-dependentylase; BROD: Benzyloxyresorufin-O-debenzyl-ase;T_4-UDP-GT: T_4-specific UDP-glucuronosyltransferase;	BROD – males: 1.34x**; females: 4.21x** HOBI-GT: males 1.23x*; females: 1.12x	

Type of data	Relevant information about the study (as applicable)	Reference
MUF-GT: 4-Methylumbeliferone-glucuronyl-transferase; HOBI-GT: 4-Hydroxy-biphenyl-glucuronyltransferase		
Microsomal P450 content Total amount of Cytochrome P_{450},	No change	

** Indicates statistically significant, $p \leq 0.01$ (Wilcoxon test)

In a two week enzyme induction study in Wistar rats, including a 4-week recovery period and employing dietary dose levels of 250, 1500, and 3000 ppm (Anonymous (2009f) – study 1 above), fluxapyroxad caused a statistically significant increase of TSH concentrations in males at 3000 ppm. A non-significant increase was noted at1500 ppm.. In contrast, T_3 and T_4 levels remained unaffected. After the 4-week recovery period no differences in TSH, T_3 or T_4 levels were observed. In females, no effects on TSH, T_3 or T_4 levels were observed. Slight increases of thyroid weights were observed in males at ≥1500 ppm although without a clear dose-response relationship. Histopathologically, as a correlate to the weight changes, follicular hyperplasia/hypertrophy was noted in males at ≥250 ppm (minimal to moderate) and in females at 3000 ppm (minimal). At the end of the 4-week recovery period no treatment related alterations of organ weights or pathological findings were observed.

Biochemical investigation of Phase II glucuronyl transferases (MUF-GT, HOBI-GT and T_4-UDP-GT) found a dose-related increase in activity. In the context of the proposed MoA, T_4-UDP-GT activity is of particular relevance as it is inducible by CAR activators, such as phenobarbitone. In the recovery group these enzyme activities mostly decreased to control levels, with exception of moderately increased MUF-GT activities in males.

A further enzyme induction study in rats using 50 ppm Fluxapyroxad (Anonymous (2010e) – study 2 above) found no effects on the thyroid or related parameters.

10.9.3.7 Perchlorate Discharge test

A single study (perchlorate discharge test) is available to investigate whether fluxapyroxad perturbs the HPT axis via inhibition of TPO.

Table 33: Summary of perchlorate discharge test in Wistar rats

<table>
<tr><th>Type of study/data
Method</th><th>Relevant information about the study (as applicable)
Observations</th><th>Reference</th></tr>
<tr><td>Perchlorate discharge test
GLP
Wistar rat (Crl:WI Han) 6/group/sex
Dietary administration
Fluxapyroxad (FPX)
99.2%
3000 ppm (283/247 mg/kg bw/day (m/f));
Positive control:
Propylthiouracil (PTU) 2000 ppm (231/192 mg/kg bw/day (m/f))
Phenobarbital (PB) 1000 ppm (89/97</td><td>
<table>
<tr><th>Group</th><th>Test Material</th><th>dietar dose (ppm)</th><th>i.p. Injection prior to necropsy</th></tr>
<tr><td>00</td><td>Control diet</td><td>0</td><td>Saline 0.9%</td></tr>
<tr><td>01</td><td>Control diet</td><td>0</td><td>Perchlorate)</td></tr>
<tr><td>10</td><td>Fluxapyroxad</td><td>3000</td><td>Saline 0.9%</td></tr>
<tr><td>11</td><td>Fluxapyroxad</td><td>3000</td><td>Perchlorate</td></tr>
<tr><td>20</td><td>Propylthiouracil</td><td>2000</td><td>Saline 0.9%</td></tr>
<tr><td>21</td><td>Propylthiouracil</td><td>2000</td><td>Perchlorate</td></tr>
<tr><td>30</td><td>Phenobarbital</td><td>1000</td><td>Saline 0.9%</td></tr>
<tr><td>31</td><td>Phenobarbital</td><td>1000</td><td>Perchlorate</td></tr>
</table>
General Toxicity
No clinical sign or mortality</td><td>Anonymous (2009g)</td></tr>
</table>

Type of study/data Method	Relevant information about the study (as applicable) Observations	Reference
mg/kg bw/day (m/f)) Prior to sacrifice (6 h) all animals received an i.p. injection of 1 µCi radiolabelled ^{125}I. Prior to sacrifice (2.5 min) each 1 of the Fluxapyroxad, PTU, and PB treated groups received an i.p. injection of either saline (0.9%) or potassium perchlorate.	Body weight: ↓ PTU bw -21 to -24% / - 10 to -13% (m/f)* **Thyroid weight** (Saline/$KClO_4$ group): ↑ Fluxapyroxad 8%15% (m), 50%/11% (f) ↑ PTU 315%/285% (m*) , 400%356% (f*); ↑ PB 46%/31% (m*), 38%11% (f) **Perchlorate discharge test** **Fluxapyroxad** ^{125}I counts in rats administered **saline** before sacrifice (% of control): Thyroid: ↑ 43/98 (m*/f); Spc. thyroid (cpm/g): ↑ 32/6 (m*/f); ^{125}I counts in rats administered **perchlorate** before sacrifice (% of control): Thyroid: ↑ 98/63 (m*/f); Spc. thyroid (cpm/g): ↑ 64/55 (m/f)*; Ratio Spc thyroid/Spc. blood: ↑ 67/48 (m*/f); **Phenobarbitone** ^{125}I counts in rats administered **saline** before sacrifice (% of control): Thyroid: ↑ 110/14 (m*/f), Spc. thyroid (cpm/g): ↑ ↑ 49/-11 (m*/f) ^{125}I counts in rats administered **perchlorate** before sacrifice (% of control): Thyroid: ↑ 102/54 (m*/f), Spc. thyroid (cpm/g):↑ 53/37 (m*/f), Ratio Spc thyroid/Spc. ↑ 48/13 (m/f) **PTU** ^{125}I counts in rats administered **saline** before sacrifice (% of control): Blood: ↑ 45/24 (m/f)*, Spc. blood (cpm/g): ↑ 45/24 (m/f)*, Spc. thyroid (cpm/g): ↓ -79/ -82, Ratio Spc thyroid/Spc. blood: ↓ -85/-86 (m/f)* ^{125}I counts in rats administered **perchlorate** before sacrifice (% of control): Blood: ↑ 48/77 (m/f)*, Spc. blood (cpm/g): ↑ 48/76 (m/f)*; Spc. thyroid (cpm/g): ↓ -91/ --80 (m/f)*; Ratio Spc thyroid/Spc. Blood, ↓ -94/-89 (m/f)*.	

* Indicates statistically significant, $p \leq 0.01$

In a thyroid function test (perchlorate discharge test; (Anonymous (2009g)), the effect of Fluxapyroxad on iodide organification after 14-day administration was investigated The basis of the test is that substances which act directly on the thyroid, can do so by inhibiting thyroid peroxidase (TPO) which causes iodide to accumulate in the thyrotrophes. Administration of perchlorate blocks further uptake of iodide from the blood stream and the accumulated iodide leaks out into the systemic circulation, which can be detected radiochemically. Propylthiouracil (PTU) was included as a TPO inhibitor and direct acting thyroid toxicant, as which inhibits TPO resulting in lower T_3 and T_4 blood levels, and a compensatory increase in TSH and TSH-mediated thyroid stimulation. Phenobarbital was included an indirect thyroid toxicant, which acts via induction of hepatic glucronyl transferases to cause TSH-mediated thyroid stimulation .

Similar to PB, treatment with Fluxapyroxad resulted in an increase of radioactive iodide in the thyroid which was not dischargeable by perchlorate, thus indicating that it does not act directly on TPO, but most likely indirectly via and increased glucuronidation and biliary excretion of T_4 followed by a compensatory TSH release from the thyroid. These data are consistent with the proposed MoA.

10.9.3.8 Early thyroid hormone changes

In a thyroid hormone study, conducted to GLP, Wistar rats (10/sex/group) were dosed with 0, 50, 250, 1500, and 3000 ppm fluxapyroxad (99.2% pure) in the diet (corresponding to 0, 3.2, 16, 96 and 192 mg/kg bw/day in males and 0, 3.8, 19, 126, 234 mg/kg bw/day in females) for 28 days. Blood was sampled at study days -3, 3, 7, 14, 21 and 28 for the determination of triiodothyronine (T3), thyroxine (T4) and Thyroid stimulating hormone (TSH).

Table 34: Summary of thyroid hormone study in Wistar rats

Type of study/data Method	Relevant information about the study (as applicable) Observations	Reference
General Toxicity	No mortality, clinical signs or effects on body weight development. Macropathology: Liver – enlarged 3000 ppm 9/10 males, 10/10 females Organ weights - liver ↑ abs ≥1500 ppm (m/f)*, rel ≥ 250 ppm (m*), ≥ 1500 ppm (f*); thyroid: ↑ abs. & rel 3000 ppm (m*)	Anonymous (2009h)
Thyroid Hormones	Initial analysis (control, 3000 ppm, all days) ↓ T_4 3000 ppm (m*) day 28 ↑ TSH 3000 ppm (m*) day 14, (f*) day 21 Final analysis (all dose levels, TSH and T_4 only) ↓ T_4 (m*) 3000 ppm day 14, 21(n.s.), 28 ↑ TSH 3000 ppm (m*) day 14	

* Indicates statistically significant, $p \leq 0.01$

Significant changes of thyroid hormone levels were restricted to increases of TSH in top dose males and females and a decrease of T_4 levels in top dose males. No statistically or biologically significant alterations of TSH and T_4 levels were noted at dose levels ≤ 1500 ppm. The hormone level changes were accompanied by significantly increased absolute and relative thyroid weights in top dose males. Statistically significant thyroid weight changes were also observed in males at 1500 ppm and in females at ≥1500 ppm.

The relevance of induction of these processes in humans is low due to differences between humans and rats. In contrast to rats, human TSH levels are more stable in response to exposure to hepatic enzyme activating agents (Dellarco *et al*, 2006[2], Meek *et al*., 2003[3]). 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

[2] Dellarco, VL, McGregor, D, Sir Berry, C, Cohen, SM, Boobis, AR (2006). Thiazopyr and thyroid disruption: Case study within the context of the 2006 IPCS human relevance framework for analysis of a cancer mode of action. Crit Rev Toxicol. 36: 793-801

[3] Meek, ME, Bucher, JR, Cohen, SM, Dellarco, VL., Hill, RN, Lehmann-Mckeeman, LD, Longfellow, DG, Pastoor, T, Seed, J. Patton, DE. (2003). A framework for human relevance analysis of information on carcinogenic modes of action. Crit Rev Toxicol. 33: 591-654

et al. 1991[4]). 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. Although the pituitary-thyroid axis in both species is responsible for the thyroid hormone homeostasis, a substantial difference exists in the dose-response relationship (Dellarco et al., 2006), with the human system being less susceptible. One further aspect to consider for determining the relevance of the rat data for humans is the shorter half-life of T4 in rats. The half-life of T4 in rats is 12 h as compared to that in the human system, which ranges between 5-9 days (Dohler *et al*. 1979[5]). The longer half-life of T4 in humans arises from the binding of T4 to a thyroid-binding globulin (Hill *et al*. 1989[6]), which is non-existent in rodents. Thus, a higher T4 turnover is present in rats as compared to humans. As a result of this higher turnover, rats have a much higher (approx. 25-fold) constitutive TSH level as compared to humans (Dohler *et al*, 1979). This means that the compensatory reaction in the rats towards a T4 deficiency is much more pronounced than in humans. This is also reflected in the histological appearance of the thyroid (Dellarco, 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. It can, therefore, be envisaged that alterations in the TSH levels can easily lead to an overstimulation of already active cells in rats resulting in the observed hypertrophy/hyperplasia, whereas in the human system it would merely activate a quiescent cell.

10.9.4 Summary and Discussion of Carcinogenicity

The carcinogenic potential of fluxapyroxad has been well investigated in standard lifetime studies in rats and mice.

10.9.4.1 Liver Tumours

Fluxapyroxad induced liver tumours in male and female rats, with the tumour incidence in males being much greater than in females. The incidence of hepatocellular adenoma and carcinoma was increased in males at ≥ 250 ppm (adenoma: 0, 0, 4, 7, 15; carcinoma: 1, 0, 1, 3, 9; out of 50 animals at 50, 250, 1500 and 3000 ppm, respectively). In females an increased incidence was noted at ≥ 1500 ppm (adenoma: 0, 2, 0, 4, 7; carcinoma: 1, 1, 0, 0, 0; out of 50 animals at 50, 250, 1500 and 3000 ppm, respectively). In males the incidences were above the historical control range at ≥250 ppm (adenoma: 0 - 4%; carcinoma: 0 - 6%).

No increases in liver tumous were noted in the mouse lifetime study.

Without knowledge of the MoA for the rat hepatocellular tumours it is not possible to establish the relevance of these tumours to humans. There are various possible mechanistic explanations that can be considered for this weak carcinogenic response in rats. They are summarised in the following table.

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[4] Curran, PG, DeGroot, LJ (1991). The effect of hepatic enzyme-inducing drugs on thyroid hormones and the thyroid gland. Endocr Rev. 12: 135-150

[5] Dohler, KD, Wong, CC, von zur Muhlen, A. (1979). The rat as model for the study of drug effects on thyroid function: Consideration of methodological problems. Pharmacol Therap, 5: 305-318

[6] Hill, RN, Erdreich, LS, Paynter, OE, Roberts, PA, Rosenthal, SL, Wilkinson, CF. (1989). Thyroid follicular-cell carcinogenesis. Fund Appl Pharmacol. 12: 629-697

Table 35: Consideration of potential modes of action for the rat hepatocellular tumours

Mode of action	**Data relating to fluxapyroxad**	**Conclusion**
Genotoxicity	Negative in standard tests	Unlikely
Cytotoxicity	No clear evidence of a cytotoxic mode of action in the liver *in vitro* or *in vivo*	Unlikely
PPARα receptor activation	No induction of CYP 4A1 gene transcription in rat hepatocytes, and there was no evidence of peroxisome proliferation (a key marker of PPARα receptor activators) in histopathological examinations.	Unlikely
CAR/PXR receptor activation	Mechanistic studies show that fluxapyroxad induces the anticipated changes in rats (see details below).	Plausible Mode of Action
AhR receptor activation	CYP mRNA induction and CYP2B enzyme activity as determined by PROD and BROD enzyme activity *in-vitro* in hepatocytes and *in-vivo* in a 14-day enzyme induction study. Some *in vitro* evidence for CYP 1AI induction with fluxapyroxad, suggesting fluxapyroxad may be have some limited AhR activating potential.	Unlikely
Porphyria	No positive evidence	Unlikely
Endocrine	No positive evidence	Unlikely
Immunosuppression	No positive evidence.	Unlikely

Recognising that fluxapyroxad may be associated with a hepatocarcinogenic effect in rats, the applicant sponsored a series of mechanistic studies (Sections 10.9.3.1-10.9.3.6) to investigate a possible non-genotoxic mode of action involving liver stimulation via an axis of constitutive androstane receptor (CAR) and pregnane X receptor (PXR) induction. As discussed in detail previously by the Risk Assessment Committee, 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 (Elcombe *et al*, 2014).

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 broadly 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. These data may indicate a lack of human relevance of the liver tumour findings seen in rats.

A critical assessment of the data is presented in the following table, with reference also to the results seen with the model substance, phenobarbital.

Key and associative events	**Evidence in rats**	**Evidence in humans**
Activation of CAR	YES. In rats suggested through the *in vitro* and *in vivo* MOA studies with increased PROD and BROD and BQ activity and increased transcription of CYP2B and CYP 3A enzymes. Potency of fluxapyroxad was lower compared to phenobarbital. CAR/KO: PROD and BROD activities were markedly decreased *in vitro*, with CAR knockout hepatocytes. Wistar/SD hepatocytes: hepatocytes from both strains of rats responded similarly, *in vitro*.	PROBABLE MOA study *in vitro* indicated increased BROD and BQ activity. Potency of BROD and BQ induction was lower compared to phenobarbital. . Induction of CYP2B mediated PROD activity was at maximum 1.8x (statistically significant) in donor 1 and 1.2x (not significant) in Donor 2. Induction of CYP2B6 mRNA was at maximum 4.41x and 3.33x in Donor 1 and 2, respectively and thus essentially comparable. Overall, these data provide some evidence for CAR activation in human hepatocytes.
Altered gene expression	YES. In rats marked increase in CYP 2B1/2 and CYP 3A3 which are controlled by CAR/PXR. Increased phase II liver enzyme transcription. The relatively minor increases in CYP 1A1, in rats are not considered to provide support for other potential MoA. CAR KO: the marked increases in gene expression were abolished. Wistar/SD hepatocytes: The same responses were observed with hepatocytes from both strains, *in vitro*.	PROBABLE In human hepatocytes there is , like in rats –, an increase of CYP2B6 (max. 4.4x in Donor 1 and max. 3.3x in Donor 2) and CYP3A4 mRNA (max. 3.7x in Donor 1 and max. 1.8x in Donor 2). The increases with Phenobarbital were 7.4x and 8.2x (CYP2B6) as well as 7.5x and 5.9x (CYP 3A3) for Donor 1 and Donor 2, respectively. No information on CYP 1A1 induction.
Hypertrophy	YES. In rats, liver hypertrophy evident in both sexes.	PROBABLE Not measured with fluxapyroxad but is predicted to occur in humans where CAR activation occurs based on published evidence in humans treated with anticonvulsant drugs.
Increased hepatocellular proliferation	YES. Observed *in-vitro* (replicative DNA-synthesis) in WT SD and Wistar rats and *in-vivo* (S-Phase response). Associative events i.e. increase of liver weights and hepatocellular hypertrophy/hyperplasia observed consistently in repeated dose studies. No induction of replicative DNA synthesis was observed *in vitro* in CAR KO hepatocytes after Fluxapyroxad treatment, indicating that the CAR nuclear receptor is essential for the induction of cellular proliferation.	NOT PREDICTED Predicted not to occur based on published evidence for species specificity of CAR activators in the literature. Evidence is available to support the lack of a proliferative effect of fluxapyroxad in humans. However, it is noted that this is limited to studies in hepatocytes from two human donors.

Altered hepatic foci	YES. At the end of the chronic phase of the combined chronic toxicity and carcinogenicity study in rats altered hepatic foci were restricted to dose levels ≤250 ppm including controls, while in all dose groups including controls 91 to 98% and 59 to 98% of the male and female survivors displayed foci of cellular alteration at end of the 2-year treatment period.	NOT PREDICTED However, there are no data.
Liver tumours	YES Statistically significant increase of tumours only observed at higher dose levels (≥ 1500 ppm) in presence of substantial cell proliferation, liver weight increase and increase severity of centrilobular hypertrophy.	NOT PREDICTED However, there are not data.

Inhibition of apoptosis and other associative events in the CAR associated tumour model have not been investigated (altered epigenetic changes, gap junctional intercellular communication and oxidative stress). However, this is not considered to be a critical knowledge gap considering the other information available.

Conclusion on liver tumours

Fluxapyroxad has been well investigated for genotoxicity, and tested negative in a battery of standard *in vitro* and *in vivo* studies. It is likely that the observed liver tumours have a non-genotoxic aetiology. The available mechanistic data indicate that the MoA for liver tumours in rats is secondary to hepatocellular proliferation induced by activation of the CAR.

It was demonstrated that the key events for this mechanism occur in the rat. 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, 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, too.

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. Therefore rat liver tumours induced by Fluxapyroxad arising via CAR activation do not appear to be relevant for human health. This is supported by the data from studies conducted with human hepatocytes, albeit only 2 human donors were used.

10.9.4.2 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 at 0, 50, 250, 1500 and 3000 ppm, respectively) and carcinoma (0, 0, 1, 1 and 3) was noted in males at 1500 ppm and 3000 ppm. While the number of follicular cell adenoma (18%) 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%, respectively 0/50 to 2/50) by one case. The incidence of follicular cell tumours in females (adenoma: 0, 3, 1, 3, 2; carcinoma: 2, 0, 1, 0, 1, 250, 1500 and 3000 ppm, respectively) was within the historical control range and not indicative of a relation to treatment. Thyroid tumours were not observed in mice.

Table 36: Consideration of potential modes of action for the rat thyroid tumours

Mode of action	Data relating to fluxapyroxad	Conclusion
Genotoxicity	Negative in standard studies	Unlikely
Cytotoxicity	No evidence of a cytotoxic mode of action in the thyroid.	Unlikely
Type 1 Deiodinase inhibition	No evidence	Unlikely
Type I1 Deiodinase inhibition	No evidence	Unlikely
TPO inhibition	Negative in a perchlorate discharge test	Unlikely
NaIS inhibition	No evidence	Unlikely
Induction of hepatic glucu-ronyltransferases	Mechanistic studies show that fluxapyroxad induces specific glucuronaly transferases responsible for hepatic clearance of the thy-roid hormones T3 and T4.	Plausible
Autoimmune disease	No evidence	Unlikely
Iodine deficiency	No evidence	Unlikely

In recognition that fluxapyroxad may cause thyroid follicular tumours in rats, the applicant spon-sored a series of mechanistic studies (Sections 10.9.3.6-10.9.3.8) 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

Information from the rat toxicodynamic studies in rats showed that fluxapyroxad activated the CAR/PXR receptor. CAR/PXR activation causes increased transcription of a specific Phase II enzyme (glucuronyl transferase) responsible for hepatic clearance of T3 and T4, eventually TSH mediated proliferation of thyroid follicular cells, and tumour induction in rats.

Thyroid follicular carcinogens can also cause tumours via two broad MoA; TSH mediated follicular cell stimulation and autoimmune disease. Those carcinogens causing thyroid tumours via TSH can be subdivided by mechanism; TPO inhibition, NaIS inhibition, inhibition of type I and II deiodinases, induction of glucuronyl transferases, and iodine deficiency.

As most of these mechanisms can be identified in short-term regulatory and mechanistic studies; data the from rat short-term, subchronic, carcinogenicity as well as several short-term MoA studies were analysed to identify the MoA of rat thyroid tumour formation and its relevance to humans.

Key and associative events	**Evidence in rats**	**Evidence in humans**
Activation of CAR	YES. In rats suggested through the *in vitro* and *in vivo* MOA studies with increased PROD and BROD and BQ activity and increased transcription of CYP2B and CYP 3A enzymes. Potency of fluxapyroxad was lower compared to phenobarbital. CAR/KO: PROD and BROD activities were markedly decreased *in vitro*, with CAR knockout hepatocytes. Wistar/SD hepatocytes: hepatocytes from both strains of rats responded similarly, *in vitro*.	PROBABLE MoA study *in vitro* indicated increased BROD and BQ activity. Potency of BROD and BQ induction was lower compared to phenobarbital Induction of CYP2B mediated PROD activity was at maximum 1.8x (statistically significant) in donor 1 and 1.2x (not significant) in Donor 2. Induction of CYP2B6 mRNA was at maximum 4.41x and 3.33x in Donor 1 and 2, respectively and thus essentially comparable. Overall, these data provide some evidence for CAR activation in human hepatocytes.
Altered gene expression	YES. In rats marked increase in CYP 2B1/2 and CYP 3A3 which are controlled by CAR/PXR. Increased phase II liver enzyme transcription. Increased CYP 1A1 in rats indicates that other potential modes of action may be possible. CAR KO: the marked increases in gene expression were abolished. Wistar/SD hepatocytes: The same responses were observed with hepatocytes from both strains, *in vitro*.	UNCERTAINPROBABLE In human hepatocytes there is , like in rats –, an increase of CYP2B6 (max. 4.4x in Donor 1 and max. 3.3x in Donor 2) and CYP3A4 mRNA (max. 3.7x in Donor 1 and max. 1.8x in Donor 2). The increases with Phenobarbital were 7.4x and 8.2x (CYP2B6) as well as 7.5x and 5.9x (CYP 3A3) for Donor 1 and Donor 2, respectively. No information on CYP 1A1 induction.
Glucuronyl transferase induction	YES. In rats T_4-UDP-GT: males: 1.1x, 1.52x**, 1.58x**; females: 1.81x, 2.38x**, 2.68x**; reversible within recovery period Other glucuronyl transferases were also induced.	NOT PREDICTED No data available

TPO inhibition	No Fluxapyroxad was investigated for TPO inhibition in rats, *in vivo*, and tested negative.	NOT PREDICTED No data available
Increased biliary clearance of T3 and T4	PREDICTED TO OCCUR No data available	NOT PREDICTED No data available
Thyroid Hormones	Yes T4 decreased and TSH increased in short-term a short-term study (up to 28-days) in rats, dosed at 192-234 mg/kg/day the highest dose tested	NOT PREDICTED No data available
Thyroid Follicular proliferation	Yes Thyroid follicular cell hyperplasia and hypertrophy with increased colloid deposition were observed in sub-acute, sub-chronic and lifetime studies in rats	NOT PREDICTED No data available
Thyroid tumours	YES Fluxapyroxad induced a slight increase in thyroid follicular cell adenomas in male rats in a lifetime dietary study. Thyroid tumours were associated thyroid cell proliferation and thyroid weight increases.	NOT PREDICTED No data available

** Indicates statistically significant, $p \leq 0.01$ (Wilcoxon test)

Conclusion on thyroid tumours

The MoA underlying the thyroid hypertrophy/hyperplasia is considered to be of no relevance to humans as indicated in Chapter 3.6.2.3.2 of the ECHA Guidance on the Application of the CLP Criteria (Version 4.1, June 2015, p. 381).

Fluxapyroxad has been well investigated for genotoxicity, and tested negative in a battery of standard *in vitro* and *in vivo* studies. Therefore, it is likely that the observed thyroid tumours have non-genotoxic aetiology. The 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 the well understood negative feedback loop. TSH-mediated stimulation of thyroid follicular cells can lead to an adaptive hyperplasic response, which if sufficiently prolonged can lead to tumour induction in these cells.

There is evidence indicating that fluxapyroxad induced a specific isoform of glucuronyltransferase (T_4-UDP-GT) responsible for hepatic clearance of thyroid hormones. However, there are no studies in bile duct cannulated animals to confirm any increase in biliary clearance. The negative findings for fluxapyroxad in the perchlorate discharge test preclude a direct effect on the thyroid via TPO inhibition. Other potential MoA; inhibition of the thyroid NaIS or inhibition of type I or type II deiodinases have not been investigated. Data from toxicodynamic studies clearly indicate that prolonged oral dosing of fluxapyroxad causes TSH-mediated stimulation of the thyroid in rats, and eventually thyroid tumours. Overall, the most plausible interpretation of the available data is

CXR/PXR induction of T_4-UDP-GT with elevated TSH levels, thyroid follicular cell hypertrophy/hyperplasia and eventual progression to follicular cell tumours. However, it is not possible to exclude contributions from some other relevant MoA (NaIS or type 1/II deiodinase inhibition).

10.9.5 Comparison with the CLP criteria

As Fluxapyroxad induced liver and thyroid tumours in rats, there is a need to consider whether classification is appropriate. There is no information from studies in humans to inform on carcinogenic potential and classification in category 1A can be excluded. Further, there is sufficient evidence to suggest that both tumour types can be dismissed as not being relevant for human health.

Fluxapyroxad has been well investigated for genotoxic potential and tested negative *in vitro* and *in vivo* therefore a genotoxic MoA can be excluded.

Liver tumours

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 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 very limited or no relevance to humans.

Thyroid tumours

Fluxapyroxad induced a specific isoform of glucuronyltransferase (T_4-UDP-GT) responsible for hepatic clearance of thyroid hormones. Data from toxicodynamic studies clearly indicate that prolonged oral dosing of fluxapyroxad causes TSH-mediated stimulation of the thyroid in rats, and eventually thyroid tumours. The most plausible interpretation of the available data is CAR/PXR induction of T_4-UDP-GT with elevated TSH levels, thyroid follicular cell hyperplasia and eventual progression to follicular cell tumours. This mechanism is not considered to be of relevance to humans.

Overall, as the observed tumours are not considered to be of relevance to humans, no classification for carcinogenicity is proposed.

10.9.6 Conclusion on classification and labelling for carcinogenicity

Not classified – Conclusive but not sufficient for classification

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 fluxapyroxad (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[7] 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, $p \leq 0.05$
** significantly different from control, $p \leq 0.01$

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.

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.

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

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 fluxapyroxad 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. 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.	Elcombe (2016a)
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	**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 acti-	Elcombe (2016d)

- high [fluxapyroxad] tested (1, 3, 10, 30 and 100 µM) - Table 28 CLH report	vator similar to PB.	
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. **d. Replicative DNA Synthesis:** 1. Fluxapyroxad did not increase the labelling index. Positive EGF control.	Elcombe (2016e)
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	**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 ↑	Anonymous (2010d)

- Table 30 CLH report	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	
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) **c. Enzyme induction:** 1. Fluxapyroxad induces significant increase in PROD and BROD (with a small level of EROD) enzyme activity indicative of CAR activation.	Anonymous (2009f)

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).

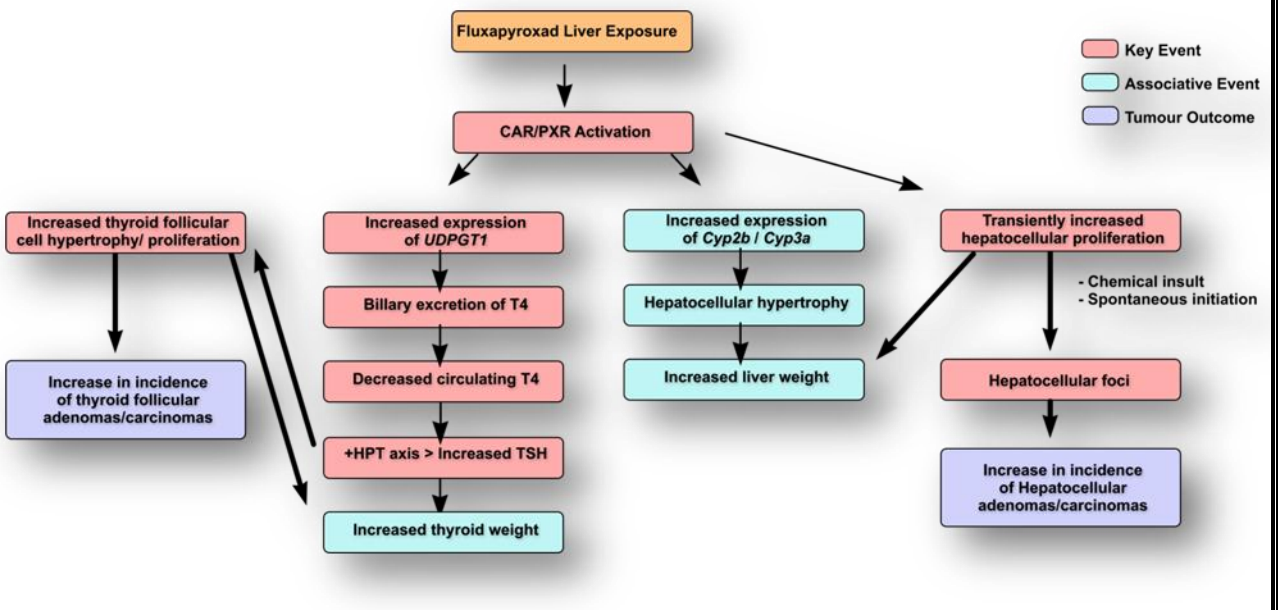

Figure: Outline of the proposed mechanisms of action for fluxapyroxad-induced tumours in rat liver and thyroid.

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 preneoplastic 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*[8]). 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*).

[8] 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.

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 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 for fluxapyroxad for carcinogenicity.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 37: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
2-generation reproductive toxicity study OECD 416 GLP Dietary, dose adjusted Rat, Han Wistar 25/sex/group in F0 and F1 generation	Fluxapyroxad Purity: 99.7% 0, 10, 50, 300 mg/kg bw/d Due to weekly dose adjustment actual dose levels were close to nominal dose levels	300 mg/kg bw/d: **<u>Parental toxicity</u>** *<u>F0 adults:</u>* No treatment-related mortality or clinical signs of toxicity ↓ FC (f*), ↓ BW <u>premating</u> 6.4%/8.2% (m/f)*, ↓ BWG 9.7%/ 19.3% (m/f)*; ↓ BW <u>gestation/lactation</u> 9.2%/6.2% (f*), BWG -14%/ +69% (f*) ↑ γ-GT (m/f)*; ↓ ALP (m*); ALT (m/f)* Organ weights: ↑ abs liver 59%/43% (m/f)*; ↑ rel liver 76%/57% (m/f)* ↑ abs thyroid 11%/10% (m/f); ↑ rel thyroid 23%/20% (m/f)* ↑ abs adrenal 11% (m*); ↑ rel adrenal 23%/9% (m/f)* In absence of histopath. changes or secondary to reduced terminal body weights (-10%/-9% (m/f)*) changes of organ weights: abs: ↓prostate (m*), ↓ pituitary (f*), ↓ spleen (f*); rel.: ↑ brain (m,/f)*, ↑ epididymides (m*), ↑ kidney (m/f)*, ↑ testes (m*),↑ ovaries (f*) Macropathology (25 animal/sex): Liver: - enlargement 23/24 (m/f), discoloration 21/23 (m/f) Incisors: - discoloration (white) 25/25 (m/f) Histopathology (25 animals/sex): Liver: - hypertrophy, centrilobular – incidence 25/25, severity: 3.2/3.3 (m/f)* - necrosis, hepatocellular - incidence 6, severity: 1.0 (m*) - cytopl. fatty vacuolation, hepatocyte - incidence: 13/5, severity: 1.0/1.0 (m*/f) Thyroid: - hypertrophy/hyperplasia, follicular, diffuse: incidence 25/25, severity: 2.0/2.0 (m/f)* - secretory depletion (altered colloid) – incidence 25/25 severity: 1.6/1.5 (m/f)* Adrenals: - cortical hypertrophy – incidence: 21/17, severity: 1.8/1.4 (m/f)* - cortical hyperplasia – incidence: 2, severity: 1.5 (m) Incisors: - absence of yellow iron-containing pigment in ameloblasts in the enamel maturation zone as well as in the enamel pigmentation zone. (special histopathological investigation of teeth from 2 representative F_0 animals/sex). No indication for 'fluorosis'.	Anonymous (2009i)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		F1 adults: No treatment-related mortality or clinical signs of toxicity ↓ FC (f*), ↓ BW premating 11%/8.9% (m/f)*, BW↓ BW gain 11% /8.4% (m/f)*; ↓ BW gestation/lactation 9.7%/7.1% (f*) BWG -11%$^{n.s.}$/+39%* (f) ↑ γ-GT (m/f)*, ↓ ALP, ALT, AST (m/f)* Organ weights: ↑ abs liver 56%/46% (m/f)*; ↑ rel liver 77%/63% (m/f)* ↑ abs thyroid 17%/13% (m*/f); ↑ rel thyroid 32%/26% (m/f)* ↑ abs adrenal 7% (m); ↑ rel adrenal 22% (m*) In absence of histopath. changes or secondary to reduced terminal body weights (-12%/-11% (m/f)*) changes of organ weights: abs: ↓ brain (m/f)*, ↓ epididymides (m*), ↓ spleen (m/f)*, ↓ testes (m*), ↓ ovaries (f*); rel. ↑ brain (m/f)*, ↑ kidney (m/f)* Macropathology (25 animal/sex): - Liver: enlargement 18/25 (m/f), discoloration 22/25 (m/f) - Incisors: discoloration 25/25 (m/f) Histopathology (25 animals/sex): Liver: - hypertrophy, centrilobular – incidence: 25/25 severity: 3.2/3.1 (m/f)* - necrosis, hepatocellular – incidence: 5, severity: 1.0 (m*) - cytopl. fatty vacuolation, hepatocyte – incidence: 7/5, severity: 1.0/1.4 (m/f) Thyroid: - hypertrophy/hyperplasia, follicular, diffuse – incidence: 25/25, severity: 2.0/2.0 (m/f)* - secretory depletion (altered colloid) – incidence: 24/25, severity: 1.4/1.4 (m/f)* Adrenals: - cortical hypertrophy – incidence: 13/10, severity: 1.5/1.2 (m/f)* Fertility and Reproductive performance No effects on oestrous cycle, sperm parameters or male/female reproductive performance were observed in F0/F1 generation. Likewise, no treatment-related mortality or clinical signs of toxicity, malformations and no effects on litter size, sex ratio or survival of pups. Offspring toxicity *F1 pups:* Lower BW from PND 1 to PND 21 PND 1: ↓ BW 10%/10% (m/f)* PND 21: ↓ BW 19%/17% (m/f)*, BW 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 secondary to lower body weight. Historical control (13 studies, 07.2000 to 02.2006): 41.5 to 45.0 days.	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Average number of days to vaginal opening not affected No treatment-related necropsy findings *F2 pups:* Lower BW from PND 7 to PND 21 PND 21: ↓ BW 12%/12% (m/f)*, BW change PND 4-21: 13%/13% (m/f)* Lower absolute and/or relative brain, thymus and spleen weights secondary to lower pup weight 50 mg/kg bw/d: Parental toxicity *F0 adults:* No treatment-related mortality or clinical signs of toxicity ↓ BW premating 6.8% (f*), ↓ BW gain -16%(f*); ↓ BW gestation/lactation 6.4%/5.0 (f*), BWG -8.2$^{n.s.}$/+51%* (f) ↓ ALT (f*) Organ weights: ↑ abs liver 23%/10% (m/f)*; ↑ rel liver 25%/19% (m/f)* ↑ rel thyroid 21%/23% (m/f)* ↑ rel adrenal 10% (f*) In absence of histopath. changes or secondary to reduced terminal body weights (-7% (f*)) changes of organ weights: abs: ↓ prostate (m*), ↓ pituitary (f*), ↓ spleen (f*); rel: ↑ brain (f*), ↑ kidney (f*), ↑ ovaries (f*) Macropathology (25 animals/sex): Liver: - enlargement 1/6 (m/f), discoloration 4/3 (m/f) Histopathology (25 animals/sex): Liver: - hypertrophy, centrilobular – incidence: 25/25, severity: 2.1/2.0 (m/f)* - cytopl. fatty vacuolation, hepatocyte - incidence: 18/8, severity: 1.1/1.0 (m*/f) Thyroid: - hypertrophy/hyperplasia, follicular, diffuse – incidence: 25/21, severity: 1.2/1.2 (m/f)* - secretory depletion (altered colloid) – incidence: 23/18, severity: 1.2/1.3 (m/f)* *F1 adults:* No treatment-related mortality or clinical signs of toxicity ↓ ALT (f*) Organ weighs: ↑ abs liver 25%/9% (m/f)*; ↑ rel liver 28%/14% (m/f)* ↑ abs thyroid 14% (m*); ↑ rel thyroid /17% (m*) In absence of histopath. changes or secondary to reduced terminal body weights (-4% (f*)) changes of organ weights: rel. ↑ kidney (m/f)*,	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		↑ brain (f*), ↑ uterus (f*) Macropathology (25 animal/sex): -Liver: enlargement 1/0 (m/f) Histopathology (25 animals/sex): Liver: - hypertrophy, centrilobular – incidence: 25/25, severity: 2.1/2.0 (m/f)* - cytopl. fatty vacuolation, hepatocyte - incidence: 14/9, severity: 1.0/1.0 (m/f)* Thyroid: - hypertrophy/hyperplasia, follicular, diffuse – incidence: 25/25, severity: 1.3/1.2 (m/f)* - secretory depletion (altered colloid) – incidence: 21/17, severity: 1.3/1.0 (m/f)* <u>Fertility and Reproductive performance</u> No effects on oestrous cycle, sperm parameters or male/female reproductive performance were observed in F0/F1 generation. Likewise, no treatment-related mortality or clinical signs of toxicity, malformations and no effects on litter size, sex ratio or survival of pups. <u>Offspring toxicity</u> <u>*F1 pups:*</u> Lower BW from PND 1 to PND 21 PND 1: ↓ BW 7.1%/7.5% (m/f)* PND 21: ↓ BW 7.3%/7.4% (m/f)*, BW change PND 4-21: 6.8%/7.1% (m/f)* No necropsy findings <u>*F2 pups:*</u> No treatment-related findings 10 mg/kg bw/d: <u>Parental toxicity</u> <u>F0 adults:</u> No treatment-related mortality or clinical signs of toxicity ↓ ALT (f*) ↑ rel liver 5% (m*) Histopathology: Liver: - hypertrophy, centrilobular – incidence: 10/5, severity: 1.0/1.0 (m*/f) <u>F1 adults:</u> No treatment-related mortality or clinical signs of toxicity ↑ abs liver 8%/3% (m/f)*; ↑ rel liver 7%/5% (m/f)* Histopathology (25 animals/sex): Liver: - hypertrophy, centrilobular – incidence 15/5, severity: 1.0/1.0 (m*/f) <u>Fertility and Reproductive performance</u>	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		No treatment-related findings Offspring toxicity *F1 pups:* No treatment-related findings *F2 pups:* No treatment-related findings% **NOAEL** (fertility/developmental/general toxicity): 10 mg/kg bw/d	

* Indicates statistically significant, $p \leq 0.01$
** Indicates statistically significant, $p \leq 0.01$ (Wilcoxon test)

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

The potential effects of Fluxapyroxad on fertility and reproductive performance have been investigated in a standard 2-generation study in the rat at doses of up to 300 mg/kg bw/d.

Parental toxicity was evident by impaired food consumption and body weight gain in females only at 50 mg/kg/day and male and female parental animals in both F0 and F1 generations at 300 mg/kg bw/day, the highest dose tested. 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, at doses of up to 300 mg/kg/day, the highest dose tested.

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. Sexual maturation of (F_1) offspring was not adversely affected by treatment. The slight delay in preputial separation in F2 pups was secondary to the slightly slower body weight development of top dose males. It is noted that preputial separation in all groups including the top dose was faster than the historical control range.

10.10.3 Comparison with the CLP criteria

Substances are classified in Category 1 where they are known to cause adverse effects on sexual function and/or fertility in humans or when there is evidence from animal studies (and other studies where relevant) to provide a strong presumption that this will be the case. Substances are classified in Category 2 where there is some evidence to this effect but is not sufficiently convincing to place the substance in Category 1.

As no human information is available regarding effects on the reproductive system by Fluxapyroxad and information from a reliable 2-generation study in rats showed that Fluxapyroxad has no effects on fertility and reproductive performance, these criteria are not met and no classification is warranted.

10.10.4 Adverse effects on development

Table 38: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Prenatal developmental toxicity study OECD 414 GLP Oral gavage Rat, Han Wistar 25 time-mated females per dose	Fluxapyroxad 99.7% 0, 25, 200, 1000 mg/kg bw/d Vehicle: 0.5% aqueous CMC Treatment: GD 6 to 19 (GD 0 = day of mating)	**1000 mg/kg bw/d:** **Maternal toxicity** No treatment-related mortality or clinical signs of toxicity ↓ FC 17%* (GD 6-8); ↓ BW change 35%* on GD 6-8 only ↑ tProt*, Alb.*, Ca^{2+}*; ↓ tBili * Organ weighs ↑ abs liver 9% *; ↑ rel liver 13% * ↑ abs thyroid 12% *; ↑ rel thyroid 16% * Histopathology: Thyroid: 7/25 hypertrophy/hyperplasia, follicular (Grade 1 – minimal) **Foetal toxicity** No treatment-related effects observed. **200 mg/kg bw/d:** **Maternal toxicity** No treatment-related mortality or clinical signs of toxicity ↓ FC 11%* (GD 6-8); ↓ BW change 25%* (GD 6-8), no significant effects on abs. BW at any interval ↑ Alb.*; ↓ tBili* ↑ abs thyroid 6% *; ↑ rel thyroid 10% *; no histopathological correlate, not dose-dependent – considered incidental **Foetal toxicity** No treatment-related effects observed. **25 mg/kg bw/d:** **Maternal toxicity** No treatment-related mortality or clinical signs of toxicity ↑ rel thyroid 9%*; no histopathological correlate – considered incidental **Foetal toxicity** No treatment-related effects observed. **NOAEL (maternal toxicity): 25 mg/kg bw/d** **NOAEL (developmental toxicity): 1000 mg/kg bw/d**	Anonymous (2009j)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Prenatal developmental toxicity study OECD 414 GLP Oral gavage Rabbit, Himalayan 25 rabbits per dose	Fluxapyroxad Purity: 99.7% 0, 10, 25, 60 mg/kg bw/d Vehicle: 0.5% aqueous CMC Treatment: GD 6 to 28 (GD 0 = day of insemi-nation)	**60 mg/kg bw/d:** **Maternal toxicity** One animal was sacrificed after abortion on GD 29. Clinical signs of toxicity. Reduced/no defecation in 9/25 does ↓ FC* (GD 8-20: decrease by 11 to 44%; GD 6 to 28: -22%), ↓ BW* (stat. sign. from GD 16 onwards; max. 6.1% lower at GD 19), ↓ BWG* (body weight loss GD 9-11, GD 6-28 49% lower) **Developmental toxicity**: ↓ Gravid uterus weight: - 25%* ↑ early resorption rate: 18.1%*, ↑ post implantation loss: 21.1%* External foetal variations: - paw hyperflexion * **25 mg/kg bw/d:** **Maternal toxicity** No treatment-related mortality or clinical signs of toxicity **Developmental toxicity**: No treatment-related effects observed. **10 mg/kg bw/d:** **Maternal toxicity** No treatment-related mortality or clinical signs of toxicity **Developmental toxicity**: No treatment-related effects observed. **NOAEL (maternal toxicity): 25 mg/kg bw/d** **NOAEL (developmental toxicity): 25 mg/kg bw/d**	Anonymous (2009k)

* Indicates statistically significant, $p \leq 0.01$

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

The potential effects of Fluxapyroxad on development have been investigated in standard studies in rats and rabbits.

In the rat, decreases of food consumption and body weight gain were observed during the first few days of treatment (GD 6-8) at 200 and 1000 mg/kg. There were no toxicologically significant changes noted in clinical chemistry or general toxicity in the dams. No treatment-related skeletal or visceral malformations or variation malformations, or retardations of development were observed at doses of up to 1000 mg/kg/day, the highest dose tested.

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 fetuses with paw hyperflexion (10.3%) at the high dose level (1000 mg/kg). This variation is frequently observed in control foetuses (up to 6.7% affected fetuses per study; 30 studies conducted

between 07/2001 and 11/2007) and is considered a minor and temporary change since tendons stretch postnatally. This is therefore of doubtful toxicological significance.

Two high dose dams were responsible for the slight but statistically significant increase of the post implantation loss and the early resorption rate, as the loss of the single implant results in a 100% early resorption/post implantation loss. The loss of single implants is common in rabbits. In this study 4 control does had one, while 3 had two resorptions. The exclusion of these two dams from calculation likewise resulted post implantation loss / early resorption values within the historical control range.

The high dose of 60 mg/kg elicited signs of maternal toxicity as indicated by reduced food consumption corroborated by an increased number of animals with reduced or no defecation, body weight loss during gestation days 9 to 11 and a decrease of cumulative body weight gain by 49% during the treatment period. No maternal toxicity was observed at dose levels ≤25 mg/kg.

10.10.6 Comparison with the CLP criteria

Substances are classified in Category 1 where they are known to cause adverse effects on development in humans or when there is evidence from animal studies (and other studies where relevant) to provide a strong presumption that this will be the case. Substances are classified in Category 2 where there is some evidence to this effect but is not sufficiently convincing to place the substance in Category 1.

There is no information available on the developmental toxicity of fluxapyroxad in humans. Information from reliable animal studies in two species showed that Fluxapyroxad has no effects on developmental properties. Therefore, the criteria for classification are not met and no classification for developmental toxicity is proposed.

10.10.7 Adverse effects on or via lactation

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

There is no indication for an effect on or via lactation.

10.10.9 Comparison with the CLP criteria

There is no indication for an effect on or via lactation.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Not classified – Conclusive but not sufficient for classification

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 hepato-biliary 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

<u>Sexual function/fertility Effects</u>

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*[9], 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.

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

Additional key elements

Table: *Rat F0 and F1 male and female mating and fertility data*

Parameter	Fluxapyroxad dose level (mg/kg bw/day)							
	F_0 generation parents				F_1 generation parents			
	0	**10**	**50**	**300**	**0**	**10**	**50**	**300**
Male fertility								
Mated [n]	25	25	25	25	25	25	25	25
Mating index [%]	100	100	100	100	100	100	100	100
Pregnant [n]	24	24	25	25	24	25	23	25
Fertility index [%]	96	96	100	100	96	100	92	100
Female fertility								
Mated [n]	25	25	25	25	25	25	25	25
Mating index [%]	100	100	100	100	100	100	100	100
Pregnant [n]	24	24	25	25	24	25	23	25
Fertility index [%]	96	96	100	100	96	100	92	100
Pre coital interval [days]	2.2	3.0	2.5	2.7	2.8	1.7	2.2	2.8
Duration of gestation [days]	22.5	22.5	**22.1****	**22.1****	22.0	22.2	21.9	21.9
Implantation sites, per dam [n]	12.3	12.6	11.8	12.0	12.0	11.9	11.1	12.0
Post implantation loss [%]	10.7	5.6	4.8	8.5	9.3	6.6	13.3	8.4
Females with live-born [n]	23	22	25	24	23	25	21	24
Gestation index [%]	96	92	100	96	96	100	91	96

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

Table: *Rat F1 and F2 birth and lactation 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
- with liveborn pups [n]	23	22	25	24	23	25	21	24
- with stillborn pups [n]	2	2	2	5	4	2	0	1
Malformed pups, observed at birth [n]	0	0	0	0	0	1	0	0
Live litter size [mean, n]	11.8	11.9	11.3	11.2	11.9	11.1	11.4	11.8
Live birth index [%]	98	99	98	97	99	98	100	100
Viability index [%]	99.6	96.5	98.6	99.6	98.9	97.4	100	98.9
Lactation index [%]	100	100	100	100	100	100	97.0	99.5
Male pup weight [mean, 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 weight [mean, 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, $p \leq 0.01$

***Table**: Rat prenatal developmental toxicity study - pregnancy and foetal data*

Parameter		**Fluxapyroxad (mg/kg bw/day)**			
		0	**25**	**200**	**1000**
Number pregnant dams	[n]	25	22	23	21
Mean corpora lutea	[n]	11.2 ± 1.57	11.5 ± 1.53	10.8 ± 1.65	11.6 ± 2.42
Mean implantation sites	[n]	10.6 ± 1.55	10.3 ± 2.23	9.8 ± 2.19	9.9 ± 1.61
Pre-implantation loss	[%]	4.6	10.9	9.7	11.1
Post-implantation loss	[%]	7.2	6.3	4.1	12.2
Mean live foetuses	[n]	9.9 ± 1.83	9.7 ± 2.17	9.4 ± 2.27	9.4 ± 1.05
Mean percent live females		44.0	47.7	54.7*	48.5
Mean placental weight	[g]	0.43 ± 0.046	0.45 ± 0.075	0.44 ± 0.148	0.43 ± 0.113
Mean foetal weight	[g]	3.4 ± 0.24	3.4 ± 0.26	3.4 ± 0.36	3.5 ± 0.24
- males	[g]	3.5 ± 0.28	3.5 ± 0.27	3.6 ± 0.38	3.6 ± 0.21
- females	[g]	3.5 ± 0.28	3.5 ± 0.27	3.6 ± 0.38	3.6 ± 0.21
Malformed foetuses					
-external, foetal incidence [n, (%)]		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
-visceral, foetal incidence [n, (%)]		0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)
-skeletal, foetal incidence [n, (%)]		0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)

***Table**: Rabbit prenatal developmental toxicity study - pregnancy and foetal data*

Parameter		**Fluxapyroxad (mg/kg bw/day)**			
		0	**10**	**25**	**60**
No. pregnant dams at terminal kill [n]		25	22	23	21
Mean corpora lutea	[n]	8.2 ± 1.38	7.3 ± 1.61	7.6 ± 1.91	7.3 ± 2.24
Mean implantation sites	[n]	7.5 ± 1.88	6.6 ± 1.80	6.8 ± 1.80	6.5 ± 2.50
Pre-implantation loss	[%]	8.3	11.3	10.3	12.4
Post-implantation loss	[%]	5.6	9.1	9.3	**21.1***
Mean live foetuses	[n]	7.1 ± 1.93	6.0 ± 2.02	6.5 ± 1.79	6.2 ± 1.78
Mean percent live females		47.2	50.7	54.2	48.3
Mean placental weight	[g]	4.5 ± 0.66	4.8 ± 0.71	4.7 ± 0.69	4.4 ± 0.60
Mean foetal weight	[g]	37.6 ± 3.86	38.8 ± 3.77	39.1 ± 3.12	35.7 ± 3.81
- males	[g]	37.6 ± 3.93	39.0 ± 4.14	39.1 ± 3.44	35.6 ± 4.99
- females	[g]	37.1 ± 4.71	38.5 ± 3.93	38.9 ± 3.07	35.9 ± 3.39
Malformed foetuses:					
-external, overall foetal incidence [n, %)]		0 (0.0)	0 (0.0)	1 (0.6)	1 (0.8)

-visceral, foetal incidence [n, (%)]	7 (4.3)	6 (4.3)	2 (1.3)	3 (2.5)
-skeletal, foetal incidence [n, (%)]	5 (3.1)	2 (1.4)	3 (1.9)	4 (3.4)
External foetal variations:				
-paw hyperflexation, foetal incidence [n, (%)]	6 (3.7)	4 (2.9)	4 (2.6)	13 (11)

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

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, $p \leq 0.01$

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

Sexual function and fertility

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.**

10.11 Specific target organ toxicity – single exposure

Table 39: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Acute toxicity, oral, OECD 423, GLP compliant Rat, Sprague-Dawley, female, 6 females/group in 2 subgroups of each 3 rats	Fluxapyroxad Purity: 99.4% 2000 mg/kg bw, single oral administration (gavage); vehicle: 0.5% aqueous CMC	LD_{50} >2000 mg/kg bw No signs of organ toxicity were observed as indicated by clinical signs of toxicity or necropsy findings.	Anonymous (2008a)
Acute toxicity, oral OECD 423, GLP compliant Rat, Wistar, male, 6 males/group in 2 subgroups of each 3 rats (Study in male rats requested by Chinese authorities)	Fluxapyroxad Purity: 99.4% 2000 mg/kg bw, single oral administration (gavage); vehicle: 0.5% aqueous CMC	LD_{50} >2000 mg/kg bw No signs of organ toxicity were observed as indicated by clinical signs of toxicity or necropsy findings.	Anonymous (2012a)
Neurotoxicity (single dose with 14-day observation period OECD 424 CLP compliant Rat, Wistar, male and female 10 animals/sex/ group	Fluxapyroxad Purity: 99.4% 0, 125, 500, 2000 mg/kg, single oral administration (gavage); vehicle: 0.5% aqueous CMC	No mortality occurred. One 125 mg/kg male displayed skin lesions at both forelimbs starting study day 7 (considered incidental). FOB at Day 0: slightly increased landing foot-splay (males, 2000 mg/kg), reduced rearing (≥500 mg/kg, males) and decreased motor activity (2000 mg/kg, both sexes). No findings at later times No (neuro-) histopathological findings	Anonymous (2009c)
Acute toxicity, dermal, OECD 402 GLP compliant Rat, Sprague-Dawley, male/female, 5/sex/group	Fluxapyroxad Purity: 99.4% 2000 mg/kg bw, single dermal application (semi-occlusive), 24 h exposure	LD_{50} >2000 mg/kg bw No signs of organ toxicity were observed as indicated by clinical signs of toxicity or necropsy findings.	Anonymous (2008b)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Acute toxicity, inhalation, OECD 403 GLP compliant Rat, Wistar, male/female, 5/sex/group	Fluxapyroxad Purity: 98.9% dust, 5.1 mg/L, head-nose inhalation, 4 h exposure MMAD: 3.3/3.4 µm GSD: 2.1/2.2	LC_{50} >5.1 mg/L Clinical signs of toxicity comprised visually increased respiration, abdominal respiration, piloerection, and squatting posture (reversible within 7 days). No signs of organ toxicity were observed based on necropsy findings.	Anonymous (2008c)

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

There were few signs of toxicity in the acute studies of Fluxapyroxad in rats. Partly reduced weight gain in females was observed, however all animals gained weight overall. In the acute inhalation study, clinical signs of toxicity comprised visually increased respiration, abdominal respiration, piloerection, and squatting posture, which were fully reversible within 7 days. No further signs of organ toxicity were observed as indicated by clinical signs of toxicity or necropsy findings. Especially, no signs of respiratory irritation were observed.

In the acute neurotoxicity study increased landing foot-splay in the 2000 mg/kg bw dose group and reduced rearing and motor activity at the day of administration were observed at ≥500 mg/kg bw. The behavioural changes were transient and minor in nature and likely represent a pharmacological response. Brain weights or neuro-histopathological examinations revealed no neuropathological findings.

No significant specific target effects were observed after oral, dermal or inhalation single exposure with Fluxapyroxad in rats.

10.11.2 Comparison with the CLP criteria

As Fluxapyroxad did not induce significant toxic effects in animal studies after single exposure and no human evidence is available, classification in Category 1 or Category 2 is not warranted. The affected clinical parameters (increased landing foot splay, reduced raring, impaired motor activity) were considered to represent an unspecific neuropharmacological effect resulting from bolus application of a high dose rather than being indicative of neuronal damage. The effects in the acute neurotoxicity study did not always display a dose-dependency or were observed only in one sex. Furthermore, the effects were fully reversed within 7 days, indicating that no significant impairment of the nervous system occurred. This assumption is supported by the fact that no treatment-related neuropathological findings were observed during histopathological examination of the nervous system. In addition, no effects on brain weight were observed. In the subchronic (dietary administration) neurotoxicity study no treatment-related effects were observed, after the first administration or during the rest of the study period.

Category 3 classification is warranted for substances that induce transient target organ effects and includes narcotic effects and respiratory tract irritation. As Fluxapyroxad did not induce significant respiratory tract irritation in animal studies after single exposure and no human evidence is available, classification in Category 3 for RTI is not warranted. The increased respiration or abdominal respiration were not considered to be severe effects. Both effects were fully reversible and no histopathological correlate was observed. Furthermore, 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.

As Fluxapyroxad did not induce (significant) narcotic effects in animal studies after single exposure and no human evidence is available, classification in Category 3 for narcotic effects is not warranted.

10.11.3 Conclusion on classification and labelling for STOT SE

Not classified – Conclusive but not sufficient for classification

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

Summary of the Dossier Submitter's proposal

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**.

10.12 Specific target organ toxicity-repeated exposure

Table 40: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
28-day study OECD 407 GLP Dietary Rat, Han Wistar 5/sex/group	Fluxapyroxad Purity: 99.1% 0, 100, 500, 2000, 6000 ppm (9, 44, 176, 530 mg/kg bw/d for males; 9, 48, 183, 531 mg/kg bw/d for females) Exposure duration 28-days	6000 ppm (530/531 mg/kg bw/d in m/f): No mortality occurred and no clinical signs of toxicity ↓ FC week 1 -13%/-19% (m/f)* ↑ WC week 3&4 34%/28% (m*) ↓ PT (f*) ↓ Cl^- (m*); ↓ ASAT (f*); ↑ γ-GT (m/f)*; ↑ Ca^{2+} (m/f)*; ↑ tProt & Glob.(m/f)*; ↑ TRIG (m/f)*; ↑ Chol. (m/f)*; ↑ iPO_4^{3-} (m*) ↑ T_3 (m*); TSH (m/f)* ↑ Urine vol. (m/f); ↓ Urine spec. gravity (m/f) ↑ abs liver wt +85%/+69% (m/f)*; ↑ rel liver wt +98%/+84% (m/f)* <u>Pathology:</u> Liver: - macropath. enlarged 5/2 (m/f) - hypertrophy, centrilob., incidence 5/5; severity 4.2/4.0 (m/f) Thyroid: - hypertrophy/hyperplasia, folic.: incidence 4, severity 1.4 (m) - altered colloid: incidence 2 (m) Femur: - Perl´s Prussian Blue stain: incidence 5/5, severity: 2.6/2.8 (m/f) 2000 ppm (176/183 mg/kg bw/d in m/f): No mortality occurred and no clinical signs of toxicity ↓ PT (f*) ↑ Ca^{2+} (m*); ↑ tProt & Glob. (m/f)*; ↑ Chol. (m/f)* ↑ TSH (m*) ↑ Urine vol. (m/f); ↓ Urine spec. gravity (m/f) ↑ abs liver wt +51%/+37% (m/f)*; ↑ rel liver wt +50%/+38% (m/f)* <u>Pathology:</u> Liver: - macropath. enlarged 2 (m) - hypertrophy, centrilob., incidence 2/2; severity 4.0/2.4 (m/f) Thyroid: - hypertrophy/hyperplasia, folic.: incidence 2, severity 1.4 (m) - altered colloid: incidence 2 (m) <u>Femur:</u> - Perl´s Prussian Blue stain: incidence: 2/2, severity: 1.0 (m&f)	Anonymous (2009l) Supplement: Anonymous (2009m)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		500 ppm (44/48 mg/kg bw/d in m/f): No mortality occurred and no clinical signs of toxicity ↑ Ca^{2+} (m*); ↑ tProt & Glob. (m*), ↑ Chol. (m*) ↑ rel liver wt 16%/12% (m/f)* <u>Pathology:</u> Liver: - hypertrophy, centrilob.: incidence 5, severity: 1.4 (m) Thyroid: - hypertrophy/hyperplasia, folic.: incidence 4, severity: 1.3 (m) - altered colloid 1 (m) 100 ppm (9/9 mg/kg bw/d in m/f): No effects observed **NOAEL**: 9/48 mg/kg bw/d (m/f) **LOAEL**:44/183 mg/kg bw/d (m/f) based on clinical chemistry changes, enlarged liver, liver weight	
90-day study OECD 408 GLP Dietary Rat, Han Wistar 10/sex/group	Fluxapyroxad Purity: 99.6% 0, 100, 500, 2000, 6000 ppm (6.1, 31, 126, 407 mg/kg bw/d for males; 7.3, 35, 144, 424 mg/kg bw/d for females) Exposure duration: 90-days	6000 ppm (407/424 mg/kg bw/d in m/f): No mortality and no clinical signs of toxicity ↓ FC (f) ↓ BW 8%/13% (m*/f*); ↓ BWG -14%/-28%) (m/f)* ↓ HGB, ↓ MCHC, ↓ PT (f*) ↓ AS(A)T (m/f)*, ↓ Cl^- (m/f)*, ↓ Gluc. (m/f)*, ↓ tBil. (m/f)*; ↑ IPO_4^{3-} (m/f)*, ↑ γ-GT (m/f)*, ↑ Ca^{2+} (m/f)*, ↑ tProt & Alb. & Glob. (m/f)*, ↑ TRIG (m/f)*, ↑ Chol. (m/f)*, Urea (m*), ↑ TSH (f*) ↑ abs liver wt 78%/57% (m/f)*; ↑ rel liver wt 99%/80% (m/f)* secondary to decreased terminal (exsanguinated) body weights (-10%/-13% (m/f)*) significant changes of absolute and/or relative adrenal, brain, heart and testes weights not accompanied by corroborative histopath were noted <u>Pathology:</u> Liver: - discoloration 10 of 10 (f) - hypertrophy, centrilobular: incidence: 10/10, severity: 2.2/3.0 (m/f) - necrosis, centrilobular, single cell: incidence: 9 severity: 1.8 (m) Thyroid: - hypertrophy/hyperplasia, follicular: incidence 8/6, severity: 1.0/1.0 (m/f) Kidney: - Pigment storage, tubular: incidence: 10, severity: 1.7 (f)	Anonymous (2009n)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
		2000 ppm (126/144 mg/kg bw/d in m/f): No mortality occurred and no clinical signs of toxicity ↓ PT (f*) ↓ Cl^- (m/f)* ↓ AS(A)T (m/f)*, ↓ Cl^- (m/f)*, ↓ Gluc. (m+), ↓ tBil. (m/f)*; ↑ IPO_4^{3-} (m*), ↑ γ-GT (m*), ↑ Ca^{2+} (m/f)*, ↑ tProt & Glob. (m/f)*, ↑ Alb. (m*), ↑ TRIG (f*), ↑ Chol. (m/f)*, Urea (m*), ↑ TSH (f*) ↑ abs liver wt 49%/41% (m/f)*; ↑ rel liver wt 50%/41% (m/f)* <u>Pathology:</u> Liver: - hypertrophy, centrilobular: incidence 10/10, severity 2.4/2.5 (m/f) Thyroid: - hypertrophy/hyperplasia, follicular: incidence 8/5, severity 1.0/1.0 (m/f) 500 ppm (31/35 mg/kg bw/d in m/f): No mortality occurred and no clinical signs of toxicity ↓ PT (f*) ↑ tBil. (m/f)*, Ca^{2+} (m*), Chol. (f*), Glob. (f*) ↑ abs liver wt 15%/13% (m/f)*; ↑ rel liver wt 18%/13% (m/f)* <u>Pathology:</u> Liver: - hypertrophy, centrilobular: incidence 9/9, severity: 1.0/1.4 (m/f) Thyroid: - hypertrophy/hyperplasia, follicular: incidence 4, severity 1.0 (f) 100 ppm (6.1/7.3 mg/kg bw/d in m/f): One low dose female died (considered incidental). No other treatment related effects were observed. **NOAEL**: 6.1/7.3 mg/kg bw/d (m/f) **LOAEL**: 31/35 mg/kg bw/d (m/f) based on clinical chemistry changes and liver wt and histopathology changes.	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
28-day study OECD 407 GLP Dietary Mouse, C57BL/6JRj 5/sex/group	Fluxapyroxad Purity: 99.1% 0, 500, 2500, 7000 ppm (112, 552, 1452 mg/kg bw/d for males; 150, 746, 2100 mg/kg bw/d for females) Exposure duration 28-days	7000 ppm (1452/2100 mg/kg bw/d in m/f): No mortality occurred and no clinical signs of toxicity ↓ BW day 7/14: -5.3%/-7.3% % (m*); BWG initial body weight loss, overall -27% (m) ↓ FC day 7: –23% (m*) ↓ WBC, ↓ HGB, ↓ HCT (m*); ↓ RBC, ↓ NEUT, ↓ Lymphocytes (m) ↑ Monocytes (m) ↓ K (m*); ↓ tProt & Alb & Glob. (m/f)*, ↓ Chol. (m/f)*, ↓ TRIG (m*/f); ↓ Urea (f*) ↑ ALP (m*/f); ↑ Na (m*); ↑ Urea (m*); ↑ abs liver wt 22%/31% (m/f)*; ↑ rel liver wt 32%/28% (m/f)* ↑ abs thymus wt 9%/50% (m/f*); rel thymus wt 18%/47% (m/f*) <u>Pathology:</u> Thymus: - hyperplasia, lymphoid, diffuse: incidence 3, severity: 1.7 (f) 2500 ppm (552/746 mg/kg bw/d in m/f): No mortality occurred and no clinical signs of toxicity ↓ tProt & Alb. & Glob. (m/f)*, ↓ Chol. (m/f)*, ↓ TRIG (m*/f), ↓ Urea (f*) ↑ abs liver wt 19%/33% (m/f)*; ↑ rel liver wt 18%/25% (m/f)* 500 ppm (112/150 mg/kg bw/d in m/f): No mortality occurred and no clinical signs of toxicity ↓ tProt & Alb. (m/f)*, ↓ Chol. (m/f)*', ↓ Glob. (f*) ↑ abs liver wt 12%/13% (m*/f); ↑ rel liver wt 9%/8% (m*/f) **NOAEL**: not observed **LOAEL**: 112/150 mg/kg bw/d (m/f) based on clinical chemistry changes	Anonymous (2009o)
90-day study OECD 408 GLP Dietary Mouse, C57BL/6JRj 10/sex/group for	Fluxapyroxad Purity: 99.6% 0, 100, 400, 2000, 6000 ppm (21, 77, 390, 1136 mg/kg bw/d for males; 32, 128, 610, 1657 mg/kg bw/d for females) Exposure duration: 90-days	6000 ppm (1136/1657 mg/kg bw/d in m/f): No mortality occurred and no clinical signs of toxicity ↓ BW -13% (m*); BWG -33% (m*) ↓ FC, overall Day 0-91: -10%/-15% (m/f)* ↑ ALP (m*), ↑ AL(A)T (m*), ↑ Urea (m*) ↓ TRIG (m*), ↓ tProt. & Alb (m/f)*, ↓ Chol. (m/f)* ↑ abs liver wt 28%/30% (m/f)*; ↑ rel liver wt 48%/28% (m/f)* secondary to decreased terminal (exsanguinated) body weight of males (-14%) significant changes of absolute and/or relative adrenal, brain, kidney and spleen weights not accompanied by corroborative histopath were noted <u>Pathology:</u> Liver: - fatty change, diffuse: increased severity 2.6 (m),	Anonymous (2009p)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		- necrosis, (multi-)focal: incidence 5 severity 1.6 (m) 2000 ppm (390/610 mg/kg bw/d in m/f): No mortality occurred and no clinical signs of toxicity ↓ TRIG (m*), ↓ Chol. (m/f)*, ↓ tProt. & Alb. (f*) ↑ abs liver wt 13%/17% (m/f)*; ↑ rel liver wt 18%/12% (m/f)* <u>Pathology:</u> Liver: - fatty change, diffuse: increased severity 2.8 (m) 400 ppm (77/128 mg/kg bw/d in m/f): No mortality occurred and no clinical signs of toxicity ↓ TRIG ((m*), ↓ Chol. (m*) <u>Pathology:</u> No findings. 100 ppm (21/32 mg/kg bw/d in m/f): No treatment-related findings **NOAEL**: 21/128 mg/kg bw/d (m/f) **LOAEL**: 77/610 mg/kg bw/d (m/f) based on clinical chemistry changes and liver weight and histopathology	
28-day study OECD 407 GLP Dietary Dog, Beagle 5/sex/group	Fluxapyroxad Purity: 99.7% 0, 2500, 7500, 20000 ppm (74, 211, 521 mg/kg bw/d for males; 85, 230, 503 mg/kg bw/d for females) <u>Exposure duration</u> 28-days	20000 ppm (521/503 mg/kg bw/d in m/f): No mortality occurred and no clinical signs of toxicity except vomiting of 5 males and 3 females predominantly during the first 3 days of treatment ↓ BW 7%/11% (m/f); BWG -149%/162% (m/f)*, i.e. overall body weight loss ↓ FC, day 0-28 -16%/-33% (m/f)* ↓ PTT (m*) ↓ tProt & Alb., ↓ Chol, ↓ tBil (f*), ↓ Ca^{2+} (m/f*); ↑ ALP, ↑ γ-GT (m/f)*,↑ TRIG (m/f)* ↑ abs liver wt 18%/10% (m/f); ↑ rel liver wt 28%/22% (m/f)* Pathology: No findings. 7500 ppm (211/230 mg/kg bw/d in m/f): No mortality occurred and no clinical signs of toxicity except vomiting of 5 males and 4 females during the first 4 days of treatment. ↓ FC, day 0 – 20 -4%/-14% (m/f)* ↓ tProt & Alb. (m/f)*, ↓ Chol (m/f*), ↓ Ca^{2+} (f*), ↓ tBil (f*) ↑ ALP (m/f)*; γ-GT (m/f*) ↑ abs liver wt 26%/13% (m/f); ↑ rel liver wt 27%/18% (m*/f) Pathology: No findings. 2500 ppm (74/85 mg/kg bw/d in m/f): No mortality occurred and no clinical signs of toxicity ↓ Alb. (m/f)*, ↓ tBil (f*) ↑ ALP (m/f)*, ↑ γ-GT (f*) Pathology: No findings. **NOAEL**: not observed	Anonymous (2009q)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		LOAEL: 74/85 mg/kg bw/d (m/f) based on clin. chem. changes	
90-day study OECD 409 GLP Dietary Dog, Beagle 5/sex/group	Fluxapyroxad Purity: 99.7% 0, 300, 1500, 10000 ppm in males (9, 45, 295 mg/kg bw/d) 0, 300, 1500, 7500 ppm in females (10, 51, 238 mg/kg bw/d) Exposure duration: 90-days	10000/7500 ppm (295/238 mg/kg bw/d in m/f): No mortality occurred and no clinical signs of toxicity except of vomiting of all animals at the first two days of treatment ↓ FC occasionally up to day 38 (m), in females throughout treatment period ↑ ALP, γ-GT (m/f)*, ↑ Trig (m*), iPO_4^{3+} (m*) ↓ tProt & Alb.(m/f)*, ↓ Chol. (m/f)*, ↓ tBil. (m/f)*; ↓ Urea (m*), ↓ Ca^{2+} (m/f)* ↑ abs liver wt 38%/17% (m/f)*; ↑ rel liver wt 34%/20% (m/f)* Pathology: No findings. 1500 ppm (45/51 mg/kg bw/d in m/f): No mortality occurred and no clinical signs of toxicity ↓ tProt. & Alb. (m/f)*, ↓ Chol. (m/f)*; ↓ Urea (m*) Pathology: No findings. 300 ppm (9/10 mg/kg bw/d in m/f): No mortality occurred and no clinical signs of toxicity No treatment-related findings. **NOAEL**: 9/10 mg/kg bw/d (m/f) **LOAEL**: 45/51 mg/kg bw/d (m/f) based on clinical chemistry changes	Anonymous (2009r)
12-months study OECD 452 GLP Dietary Dog, Beagle 5/sex/group	Fluxapyroxad Purity: 99.4% 0, 300, 1500, 12000 ppm in males (8, 39, 335 mg/kg bw/d) 0, 300, 1500, 9000 ppm in females (9, 43, 257 mg/kg bw/d) Exposure 12 months	12000/9000 ppm (335/257 mg/kg bw/d in m/f): No mortality occurred and no clinical signs of toxicity except vomiting at multiple occasions through the study period (m/f) ↓ FC day 1 to365 -18% (f*), occasionally significant differences in male, overall FC only 1% lower than control ↓ BW -9%/-10% (m/f); ↓ BWG -33%/-42% (m/f*) ↑ PLT (m/f)* ↑ ALP (m/f)*, ↑ γ-GT (m/f)*, ↑ AL(A)T (m*), ↑ TRIG (m*) ↓ tProt &Alb. (m/f)*, ↓ Chol. (m/f)*, ↓ Ca^{2+} (m/f)*, ↓ Urea (m/f), ↓tBil. (m/f)*, ↓ Crea (m*) ↑ abs liver wt 24%/15% (m/f)*; ↑ rel liver wt 35%/23% (m/f)* ↓ abs/rel prostate wt 64%/61% (m*) ↓ abs spleen wt 31%/28% (m*/f); ↑ rel spleen wt 24%/23% (m*/f) <u>Pathology:</u> Liver: - discoloration, brownish: incidence 5/5 (m/f) (macropath. observation) - iron stain (Fe^{3+}), hepatocytes: incidence 5/5, severity: 3.0/1.8 (m/f) - fibrosis, multifocal: incidence 4/3, severity: 2.0/1.0 (m/f) - cirrhosis, diffuse: incidence 1, severity 2.0 (m)	Anonymous (2009s)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
		Spleen: - "deposition" – diffuse grey-white irregular thickening of the capsule: incidence 5/5 (m/f) (macropath. observation) - iron stain (Fe^{3+}), connective tissue: incidence 5/5, severity 3.4/3.0 (m/f) - atrophy, diffuse, red pulp: incidence 4/3, severity: 2.8/1.7 (m/f) Gall bladder: - pigment storage (origin unknown): incidence 2/4 severity: 1.5/1.3 (m/f) Prostate: - atrophy, diffuse (related to the reduced size, the histopathological appearance of the gland was not changed): incidence 5, severity: 2.6 (m) 1500 ppm (39/43 mg/kg bw/d in m/f): No mortality occurred and no clinical signs of toxicity ↑ PLT (f*) ↑ ALP (m/f) ↓ tProt & Alb. (m/f)*, ↓ tBil. (m/f)*, ↓ Chol. (m*), ↓ Urea (m*), ↓ Ca^{2+} (m*) <u>Pathology:</u> Liver: - iron stain (Fe^{3+}), hepatocytes: incidence 4/2, severity: 1.0/1.0 (m/f) Spleen: - iron stain (Fe^{3+}), connective tissue: incidence 4/2, severity 1.3/1.0 (m/f) <u>Gall bladder:</u> - pigment storage (origin unknown): incidence 4/3 , severity: 1.0/1.5 (m/f) 300 ppm (8/9 mg/kg bw/d in m/f): No treatment-related findings. **NOAEL**: 8/9 mg/kg bw/d (m/f) **LOAEL**: 39/43 mg/kg bw/d (m/f) based on clinical chemistry changes	
90-day neurotoxicity OECD 424 GLP Dietary Rat, Han Wistar 10/sex/group (5 animals/sex perfusion fixed, 5 animals/sex	Fluxapyroxad Purity: 99.4% 0, 200, 1000, 5000 ppm (11.5, 58 and 302 mg/kg bw/d in males; 13.4, 67, 338 mg/kg bw/day in females) Exposure duration: 90-days	5000 ppm (302/338 mg/kg bw/d in m/f) No mortality or clinical signs of toxicity, except of incisor whitening in all rats No treatment-related FOB of motor activity findings ↓ BWG -21% (f*) ↑ γ-GT (m*), ↑ tProt & Alb. & Glob. (m/f)*, ↑ Chol (m/f)*, ↑ Ca^{2+} (m/f)*, ↑ iPO_4^{3-}(m*), Mg^{2+} (f*), ↑ urea (f*), ↑ creatinine (f*) ↓ AS(A)T (f*), ↓ tBil (f*) ↑ abs liver wt 85%/71% (m/f)*; ↑ rel liver wt 78%/80% (m/f)* ↑ abs thyroid wt 50%/61% (m/f)*; ↑ rel thyroid wt 43%/71%	Anonymous (2009t) 1. Amendment Anonymous (2009t) 2. Amendment Anonymous (2009t) 3. Amendment Anonymous (2009t)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
normal fixation)		(m/f)* No neurohistopathological findings Liver (non perfusion fixed animals): - enlarged: incidence 5/5 - hypertrophy, centrilobular: incidence 5/5, severity 3.8/4.0 (m/f) 1000 ppm (58/67 mg/kg bw/d in m/f) No mortality or clinical signs of toxicity No treatment-related FOB of motor activity findings ↑Glob. (m/f)*, ↑ Chol (f*), ↑ iPO_4^{3-}(m*),↑ urea (f*), ↑ creatinine (f*) ↓ AS(A)T (f*), ↓ tBil (f*) ↑ abs liver wt 35%/24% (m/f)*; ↑ rel liver wt 27%/24% (m/f)* ↑ abs thyroid wt 39%/50% (m/f)*; ↑ rel thyroid wt 27%/53% (m/f*) No neurohistopathological findings Liver (non perfusion fixed animals): - enlarged: incidence 4/5 - hypertrophy, centrilobular: incidence 5/5, severity 2.0/3.0 (m/f) 200 ppm (11.5/13.4 mg/kg bw/d in m/f) No mortality or clinical signs of toxicity No treatment-related FOB of motor activity findings ↑ abs thyroid wt 25%/31% (m/f)*; ↑ rel thyroid wt 18%/39% (m/f*) No neurohistopathological findings Liver (non perfusion fixed animals): - hypertrophy, centrilobular: incidence 5, severity 1.2 (m) Neurotoxicity: **NOAEL**: 302/338 mg/kg bw/d (m/f) **LOAEL**: not achieved Systemic toxicity: **NOAEL**: not achieved **LOAEL**: 11.5/13.3 mg/kg bw/d (m/f)	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
28-day immunotoxicity study OPPTS 870.7800 GLP Dietary Mouse, C57BL/6 J Rj 8 males/dose	Fluxapyroxad Purity: 99.2% 0, 500, 2000, 6000 ppm (106, 450, 1323 mg/kg bw/d) Exposure duration: 28-days Positive control Cyclophosphamid 12 mg/kg Administered daily by oral gavage (vehicle water)	6000 ppm (1325 mg/kg bw/d) No treatment-related effects on any investigated parameter 2000 ppm (450 mg/kg bw/d) No treatment-related effects on any investigated parameter 500 ppm (106 mg/kg bw/d) No treatment-related effects on any investigated parameter Cyclophosphamide (positive control) 12 mg/kg bw/day ↓ BW -9.5%*, ↓ BWG -77%* ↓ total Lymphocyte count* ↓ abs. and rel. B- and T-lymphocyte count and ratio* ↓ abs. T_{Helper} and $T_{Cytotoxic}$ lymphocyte count* ↓ abs, and rel. Natural Killer cell counts* ↓ anti sheep IgM* ↓ abs. spleen/thymus wt -16%/-60%, rel. spleen/thymus wt -13%/-56% **NOAEL**: 1325 mg/kg bw/d **LOAEL**: not achieved	Anonymous (2009u)
28-day study OECD 410 GLP Dermal, semi-occlusive (6 h/d) Rats 10/sex/group	Fluxapyroxad Purity: 99.2% 0, 100, 300, 1000 mg/kg bw/d Exposure duration: 28-days on a 5d per week basis	1000 mg/kg bw/d in m/f: No mortality occurred and no treatment-related clinical signs of toxicity ↑ tProt & Glob. (m*), change within historical control range ↑ abs liver wt 14%/14% (m/f)*; ↑ rel liver wt 14%/10% (m/f)* Pathology: No treatment related findings. 300 mg/kg bw/d in m/f: No mortality occurred and no clinical signs of toxicity ↑ tProt & Glob. ($m^{n.s.}$), change within historical control range Pathology: No findings. 100 mg/kg bw/d in m/f: No mortality occurred and no clinical signs of toxicity were observed. ↑ tProt & Glob. (m*), change within historical control range Pathology: No findings. **NOAEL**: 300 mg/kg bw/d (m/f) **LOAEL**: 1000 mg/kg bw/d (m/f)	Anonymous (2009v)

* Indicates statistically significant, $p \leq 0.01$

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

Repeated dose studies were conducted in rats (28-day, 90-day, and 2-year), mice (28-day, 90-day, 18-months) and dogs (28-day. 90-day, 1-year). Additional information regarding repeated exposure toxicity in available from a 2-generation toxicity study in rats and a sub chronic (90-day) neurotoxicity study. A summary of the 2-year rat study and the 18-month mouse study is provided in table 24 in section 10.9. A summary of the 2-generation study in the rat is provided in table 38 in section 10.10.

Studies in rats: Dietary administration of Fluxapyroxad for 28 days in the rat at concentrations of 44-48 mg/kg/day and above in males caused adverse effects in the liver (increased liver weight and centrilobular hepatocyte hypertrophy) and thyroid (follicular hypertrophy/hyperplasia, altered colloid). Similar changes in the liver were elicited in females, at concentrations of 176-183 mg/kg/day and above. The effects in the liver and thyroid were accompanied by minor clinical chemistry changes and, at,176-183 mg/kg/day increased TSH levels. Additionally, changes in bones were present at 176-183 mg/kg/day, namely the deposition of ferric iron in the femur, though the toxicological significance of this finding is unclear.

Dietary administration of Fluxapyroxad for 90 days in the rat at concentrations of 31-35 mg/kg/day and above caused adverse effects in the liver (increased liver weight and centrilobular hepatocyte hypertrophy) and thyroid (follicular hypertrophy/hyperplasia). The effects in the liver and thyroid were accompanied by minor clinical chemistry changes and, in females only, increased TSH levels. Additionally, at the highest dose level, single cell necrosis was observed in males and there was an increased incidence and severity of intracytoplasmic storage of yellow-brownish pigment in the proximal tubular epithelium of the kidneys in females.

Regarding the non-neoplastic effects in the 2-year combined chronic toxicity and carcinogenicity study of Fluxapyroxad, the liver, thyroid glands, bone, teeth and haematological system are identified as targets. Concentrations of ≥250 ppm caused increased liver weight and hepatocellular hypertrophy, iron disposition in the femur, tooth whitening, decreased clotting time, occasional clinical chemistry changes and reduced bodyweight gain. Additionally, at higher concentrations, Fluxapyroxad caused spongiosis hepatis, thyroid follicular cell hyperplasia, hyperostosis of skull bones, and reduced MCH and MCV.

Dietary administration of Fluxapyroxad over two generations caused general toxicity in the F_0 and F_1 parental generations at 50 and 300 mg/kg/day, observed as reduced body weight gain and food consumption, liver changes (increased weight, centrilobular hepatocellular hypertrophy and hepatocellular necrosis (300 mg/kg bw/d only) and thyroid changes (follicular hypertrophy/ hyperplasia and secretory depletion). Additionally, only at 300 mg/kg/day, elevated plasma γ-GT levels and tooth whitening were observed.

In conclusion; independent of the exposure duration, Fluxapyroxad affected body weight development at higher doses and caused comparable effects in the target organs (liver, thyroid, bone, teeth). In the liver a dose dependent increase of weights accompanied by centrilobular hepatocyte hypertrophy and associated changes of clinical chemistry parameters. Degenerative changes in the liver (hepatocellular single cell necrosis) were restricted to dose levels ≥300 mg/kg in the 90-day study and the 2-generation study. Increased thyroid weights were not always strictly dose dependent. However, hypertrophy/hyperplasia of the follicular epithelium, often associated with an alteration/depletion of colloid, was noted consistently. The deposition of ferric iron (Fe^{3+}) in the femur (the only long bone investigated) was observed in the 28-day and the 2-year study (femur not investigated in the 90-day study) without an increase of incidence or severity. There were no

obvious adverse effects of this iron storage. This holds true for the whitish discoloration of the incisors observed at higher dose levels in studies of 28-days to 2-year duration. This discoloration was due to the loss of an iron containing pigment in the outer enamel of the teeth, which was without any adverse effect on function of the incisors.

Studies in mice: Dietary administration of Fluxapyroxad for 28 days to the mouse at concentrations of 112-150 mg/kg/day and above caused adverse effects, observed as reductions in total protein, albumin and cholesterol levels in both sexes. At 552-760 mg/kg/day, adverse effects were apparent in the liver, observed as increased organ weight. At the highest dose level tested, 1452 – 2100 mg/kg/day, the highest dose tested, some haematology changes were present in males (WBC increased, haemoglobin levels and haematocrit reduced) and hyperplasia of the thymus was seen in some females.

Dietary administration of Fluxapyroxad for 90 days to the mouse at concentrations of 77 – 128 mg/kg/day and above caused adverse effects observed as a decrease in triglyceride and cholesterol levels in males. At 390-610 mg/kg/day and above adverse effects were apparent in the liver, observed as increased organ weight. At the highest concentration tested, bodyweights and food consumption were reduced and there were clinical chemistry changes probably related to the increased liver weight.

Regarding the non-neoplastic effects in the 18-months carcinogenicity study, the liver is identified as the principal target. Fluxapyroxad caused increased liver weight at concentrations of ≥150 ppm and the incidence and severity of (partially macrovesicular) fatty change and hepatocellular hypertrophy were increased at higher concentrations. The effects on the liver were accompanied by minor clinical chemistry changes and reduced bodyweight gain at the higher concentrations. Also, tooth whitening occurred in a number of animals at males and females at ≥750 ppm.

In conclusion, independent of the exposure duration, Fluxapyroxad at higher doses affected the body weight development. The liver was the principal target organ as indicated by increased liver weights, hepatocellular hypertrophy fatty change (vacuolation) and changes of liver related clinical chemistry parameters. Hepatocellular necrosis or (slight) increases of ALT were restricted to dose levels >1000 mg/kg bw/day.

Studies in dogs: Dietary administration of Fluxapyroxad for 28 days to the dog at concentrations of 74 – 85 mg/kg/day and above caused liver related clinical chemistry changes (increased ALP, γ-GT,). At the higher concentrations there was a treatment related increase in liver weight and reduced body weight gain.

Dietary administration of Fluxapyroxad for 90 days to the dog at concentrations of 45 – 51 mg/kg/day and above caused clinical chemistry changes (increased ALP, γ-GT, triglyceride and PO_4^-, decreased protein, albumin and cholesterol, bilirubin and urea, possibly related to effects on the liver). At the highest concentration tested there was a treatment related increased liver weight and reduced bodyweight gain.

Dietary administration of Fluxapyroxad for 12 months to the dog caused adverse effects in the liver, spleen and prostate. Effects in the liver were present at 39 - 43 mg/kg/day and above, observed as iron staining of hepatocytes and, in females, fibrosis; at the highest concentrations tested (335 mg/kg/day in males and 257 mg/kg/day in females), liver weight was increased and cirrhosis was present in one female. Minor clinical chemistry changes (increased ALP, γ-GT and ALAT, decreased total protein and albumin), possibly related to the effects on the liver, and was present at 1500 and 9000/12000 ppm. Regarding the spleen, iron staining of the connective tissue was present at 1500 ppm and above and the organ weight was increased at the highest concentration. Prostate

weight was markedly lower at the highest dose, an observation corroborated by the presence of histopathologically diagnosed atrophy in this organ in all animals in this group.

In conclusion, high doses (≥9000 ppm, ~ ≥250 mg/kg bw/d) of Fluxapyroxad resulted in impaired body weight development. Even though liver weight increases were observed already after 28-days of treatment, histopathological changes of the liver (and other organs) were observed after 1-year of treatment only. These consisted of Fe^{3*} deposition in hepatocytes, fibrosis and cirrhosis at ≥ 260 mg/kg bw/d. Deposition of Fe^{3*} was also noted in the spleen at ≥~40 mg/kg bw/d, which was marked at ≥260 mg/kg and accompanied by a diffuse atrophy of the red pulp. The substantial decrease of prostate weights at 335 mg/kg bw/d to about one third of the control weights, which was histopathologically described as 'atrophy', was not accompanied by histopathological changes of the glandular tissue, indicating a normal function of the prostate.

10.12.2 Comparison with the CLP criteria

At dose levels that would merit classifications as STOT-RE (≤ 100 mg/kg bw/day based on a 90-day study in the rat and amended as required to account for the duration of the study), Fluxapyroxad caused a variety of effects on haematological and clinical chemistry parameters, organ weights and histopathology of the main target organ, i.e. the liver, in rats, mice and dogs. However, the changes observed at ≤100 mg bw/d were adaptive and do not represent significant adverse toxicological effects. Thus, it is considered that the criteria for classification are not met and classification as STOT-RE is not warranted.

10.12.3 Conclusion on classification and labelling for STOT RE

Not classified – Conclusive but not sufficient for classification

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

Summary of the Dossier Submitter's proposal

The DS 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 **Females**: 230 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) **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)	DAR B.6.3.3 (IIA 5.3.1/4)
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	≤ 1.67	≤ 16.7	**Males**: No relevant dose to compare with criteria. **Females**: No relevant dose to compare	DAR B.6.5.2 (IIA 5.5.3/1)

	Females: 33 mg/kg bw/day			with criteria.	
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)	DAR B.6.6.1 (IIA 5.6.1/1)

				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) **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**.

10.13 Aspiration hazard

Not relevant for solid substances.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Fluxapyroxad (often referred to in test reports as BAS 700 F) is a fungicide used in the control of plant pathogenic fungi. Available environmental fate and hazard studies have been considered under Directive 91/414/EEC and summarised in the Draft Assessment Report (DAR) 2011.

The key information pertinent to determining a classification is presented below.

11.1 Rapid degradability of organic substances

Table 41: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Test type: ready biodegradability Supernatant from sedimented activated sludge; inoculum from municipal sewage treatment plant DIN-EN 29439 (1993) equivalent or similar to OECD Guideline 301 B (Ready Biodegradability: CO_2 Evolution Test) GLP	% Degradation of test substance: < 10 % CO_2/ThCO_2 after 28 d (CO_2 evolution) Not readily biodegradable	Test material: Fluxapyroxad	Schwarz, 2008
Test type: hydrolysis Sterile aqueous buffer solutions at environmentally relevant pH values (i.e. pH 4, 5, 7 and 9) OECD guideline 111 GLP	Stable in water under environmentally relevant acidic, neutral and alkaline conditions. <10% hydrolysis observed DT_{50} at 25ºC >1year	Test material: Fluxapyroxad	Hassink, 2009a
Test type: water/sediment study Two different water/sediment systems (Berghäuser Altrhein and Ranschgraben), incubation under dark and irradiated conditions OECD 308; EPA 162-4; EPA 835.4300 GLP FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration	Dark experiment converted to 12ºC: $DT_{50,whole\ system}$: 1316 to >1896 days $DT_{50,\ water}$: 6.4 to 9.7 days (dissipation) $DT_{50,sediment}$: could not be calculated Irradiated experiment converted to 12ºC: $DT_{50,whole\ system}$: 355 to 444 days $DT_{50,\ water}$: 6.4 to 13.3 days (dissipation) $DT_{50,sediment}$: 225 to 328 days Two metabolites appeared at concentrations > 5% Total Applied Radioactivity: M700F001 and M700F007	Test material: [pyrazole-4-^{14}C]-labelled and [aniline-U-^{14}C]-labelled Fluxapyroxad	Ebert, 2009

Method	Results	Remarks	Reference
Test type: aqueous photolysis Sterile aqueous buffer at pH 7 and 22°C According to FAO Revised Guidelines on Environmental Criteria for the Registration of Pesticides Revision 3 (28 August 1993); EEC 94/37; EEC 91/414; EPA 161-2; JMAFF No 12 Nosan No 8147; SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995); OECD Guideline Proposal - Phototransformation of Chemicals in Water - Direct Photolysis (December 2007) GLP	Fluxapyroxad is stable in water at pH 7 with and without influence of light.	Test material: [pyrazole-4-^{14}C]- and [aniline-U-^{14}C]-labelled BAS 700 F (purity: 98.2-98.6%)	Hassink, 2009b
Test type: aqueous photolysis sterile natural water at 22°C According to FAO Revised Guidelines on Environmental Criteria for the Registration of Pesticides Revision 3 (28 August 1993); JMAFF No 12 Nosan No 8147; EEC 91/414; EEC 94/37 GLP	Test using aquatic degradant M700F007. M700F007 is stable in sterile natural pond water at pH8 with and without influence of light.	Test material: M700F007 (purity: 99.4%)	Hassink, 2009c

11.1.1 Ready biodegradability

A carbon dioxide evolution test (former Sturm test) has been conducted to assess the rapid biodegradability of Fluxapyroxad (as BAS 700 F). At test termination after 28 days results show that Fluxapyroxad was not mineralized to carbon dioxide. In fact, less than 10% CO_2/ThCO_2 was detected. A study summary is provided below.

Author(s)	Schwarz H.
Year:	2008
Title:	BAS 700 F: Determination of the biodegradability in the CO_2-evolution test
Test Guidelines:	OECD 301 B; EEC 92/69; ISO 9439; EPA 835.3110
Deviations from Guideline:	No
GLP Compliance:	Yes

The aerobic biodegradability of Fluxapyroxad was investigated in water containing mineral salts and microbial inoculum. Municipal activated sludge from the wastewater treatment plant of Mannheim/Baden-Württemberg (Germany) was used as biological test system. A suitable aliquot of the activated sludge suspension was sieved by a finely woven mesh with a mesh size of about 1 mm. After settling, the supernatant was discarded and the sludge suspension was filled up with tap water. The sludge of the suspension was adjusted to a concentration of 6.0 g/l dry weight and then added to the test vessels to obtain a sludge concentration of 30 mg/l dry substance. The activated sludge suspension in the test vessels was pre-aerated for about 24 hours in the dark at a temperature of 22±2°C. Fluxapyroxad was added to the test medium and activated sludge inoculum to achieve a concentration of 10 mg TOC/L corresponding to approximately 18 mg/l Fluxapyroxad. The blank control assays contained only mineral medium and activated sludge. The assay for inhibition control contained the test substance and the reference substance in the same concentration in relation to its total organic carbon content.

Duplicate control systems containing the microbial inoculum without test or reference substance were used to determine the endogenous microbial CO_2 evolution. Duplicate inoculated test substance systems dosed with the test substance at a nominal concentration of 10 mg TOC/L, were used to monitor biodegradation of the test substance. A reference substance system containing readily biodegradable aniline at a nominal concentration of 20 mg TOC/l was also tested to verify the viability of the microbial inoculum. All systems were sampled on days 1, 3, 7, 9, 14, 16, 20, 22, 24, 27, 28 and 29. The average CO_2 evolved from the control systems was subtracted from the CO_2 evolved in the test and reference substance systems.

The test substance systems yielded theoretical carbon dioxide ($ThCO_2$) values of <10% $CO_2/ThCO_2$ after an exposure period of 28 days (mean value from two single test assays). Therefore, Fluxapyroxad was not biodegradable under the conditions of the test. Biodegradation in the reference substance system reached 88 % $CO_2/ThCO_2$ at the end of the study verifying that the microbial inoculum was viable and active.

The percent theoretical CO_2 produced by the test substance Fluxapyroxad was <10% $ThCO_2$ after 28 days of incubation. Therefore, Fluxapyroxad is considered not readily biodegradable.

11.1.2 BOD_5/COD

For the purpose of classification, data generated by the ready biodegradability study supersede direct BOD_5 and COD measurements.

11.1.3 Hydrolysis

A preliminary hydrolysis test at 50°C over 5 days at pH 4, 5, 7 and 9 has been conducted according to OECD Guideline 111 with Fluxapyroxad. No hydrolysis of Fluxapyroxad was observed and the test substance was found to be stable in aqueous solution under sterile conditions. Thus, the main test at 25°C was not performed.

Author(s)	Hassink J.
Year:	2009(a)
Title:	BAS 700 F: Aqueous hydrolysis at four different pH values
Test Guidelines:	According to EPA 161-1; EEC 94/37; EEC 95/36; JMAFF No 12 Nosan No 8147; OECD 111; EEC 91/414; SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995)
Deviations from Guideline:	No
GLP Compliance:	Yes

The hydrolytic stability of [pyrazole-4-^{14}C]- Fluxapyroxad was investigated in sterile aqueous buffer solutions at four environmentally relevant pH values and at a concentration of about 1 mg/l. According to OECD Guideline 111 the test was performed for up to 5 days at 50°C in the dark at pH 4, 5, 7 and 9 to investigate if the test item is hydrolytically stable.

The sterile samples (25 ml subsets) were stored in a climatic chamber at 50°C in the dark. Sampling was performed immediately after treatment (time 0) and after 1, 2, 3, 4 and 5 days of incubation. All samples of the test solutions were analysed without a work-up by high performance liquid chromatography. The material balances for the incubations were 100.0 - 104.0% (pH 4), 99.9 - 102.2% (pH 5), 100.0 - 102.3% (pH 7) and 100.0 - 104.8% (pH 9) of the initially applied amount.

Fluxapyroxad was hydrolytically stable at all four pH values, representing 101.5%, 101.0%, 101.2% and 102.0% (mean recoveries) for pH 4, 5, 7 and 9, respectively at the end of the 5-day incubation period. Since there was less than 10% hydrolysis of the test item over the 5-day period for all pH values, there was no need for a main test at 25°C. Due to the hydrolytical stability of the test item, no half-lives or DT_{90} values were calculated.

From the results of the hydrolysis study with Fluxapyroxad conducted using sterile buffer solutions at 50°C, it can be concluded that Fluxapyroxad is stable in water under environmentally relevant acidic, neutral and alkaline conditions. Given that less than 10% hydrolysis was observed, the hydrolysis DT_{50} at 25°C is considered >1year.

11.1.4 Other convincing scientific evidence

No data.

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

No data.

11.1.4.2 Inherent and enhanced ready biodegradability tests

Not available for Fluxapyroxad.

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

11.1.4.3.1 Water-sediment study

The degradation of Fluxapyroxad (tested as ^{14}C-fluxapyroxad) in aerobic water/sediment systems was investigated under dark and irradiated conditions. Two different natural water/sediment systems were used for incubation in both experiments. The systems were treated with pyrazole-4-^{14}C-labeled and aniline-U-^{14}C-labeled Fluxapyroxad, respectively.

Author(s)	Ebert D.
Year:	2009(a)
Title:	Degradation of BAS 700 F in water/sediment systems under aerobic conditions
Test Guidelines:	According to OECD 308; EPA 162-4; EPA 835.4300
Deviations from Guideline:	No
GLP Compliance:	Yes

The degradation of ^{14}C- Fluxapyroxad in aerobic water/sediment systems was investigated under dark conditions and irradiated conditions.

Two different natural water/sediment systems were used for incubation in both experiments. One system was taken from a pond like side-arm of a river (Berghäuser Altrhein) with a silty loam sediment. The second system was taken from a small stream (Ranschgraben) surrounded by a forest with a sandy sediment.

The systems were treated with [pyrazole-4-^{14}C]-labelled and [aniline-U-^{14}C]-labelled Fluxapyroxad, respectively dissolved in acetone which is a noted photo-sensitiser. About 9 – 10 µg of ^{14}C-Fluxapyroxad was applied to each of the test vessels containing about 300 ml of water and about 180 g of wet sediment. In the dark experiment the sediment:water ratio was 2.5:6. In the irradiated experiment the sediment:water ratio was 1:3. The influence of microbial activity was tested during the dark experiment by applying the test substance to sterilized vessels.

For the experiment in the dark, the test vessels were attached to a flow through system for continuous aeration and incubated at a temperature of 20°C for 100 days. For the experiment under irradiated conditions, the test vessels were also connected to an aeration system and placed in a climatic chamber (phytotron) providing a uniform day/night cycle with 13 hours light (constant light intensity of about 28 kilolux) and 11 hours dark for 57 days. The temperature control of the climatic chamber was appropriately programmed so that the temperature in the test vessels could be kept in a range of about 22-26°C during daylight and 18-20°C during night.

Samples for the dark experiment were taken at 0, 1, 3, 7, 14, 30, 62, and 100 days after treatment (DAT). Since two radio-labels were tested separately, they can be considered as replicates for the degradation results of the test item. Samples for the irradiated experiment were taken at 0, 1, 4, 7, 14, 29, 43, and 57 days after treatment. The two tested labels were considered as replicates.

Water samples and sediment extracts were analysed by radio-HPLC. The amount of non-extractable residues was determined by combustion and liquid scintillation counting (LSC). Volatiles were trapped

in appropriate trapping solutions and also analysed by LSC. Metabolite identification was done by co-chromatography with reference substances and by HPLC-MS/MS analysis.

Under dark conditions, the radioactivity in the water decreased from initially 87-94% TAR (Total Applied Radioactivity) to 9-14% AR after 100 days. Correspondingly, the radioactivity in the sediment increased in both systems reaching 84-87% TAR at the end of the incubation. After 100 days, fluxapyroxad was found in the water at levels of 8-9% TAR in system Berghäuser Altrhein and 13-14% TAR in system Ranschgraben. Metabolite M700F02, a cleavage product of fluxapyroxad consisting only of the pyrazole-moiety, was found in system Berghäuser Altrhein approaching 4% TAR towards the end of incubation. No other metabolite ever exceeded 2.1% TAR. The sediment analyses show that fluxapyroxad reached its highest amount after 100 days with 74-77% TAR. Almost no degradation products were found in sediment extracts except one metabolite sporadically occurring with the aniline-label at maximum 2.1% TAR. The material balance in the test vessels ranged between 96.1% and 101.5% of the total applied radioactivity (TAR).

Under irradiated conditions, the degradation slightly differed from the dark experiment. In the pyrazole-label experiments, metabolites M700F001 as well as M700F007 ("amide") were formed in both systems (max. amounts of 11 and 7.5% TAR, respectively). MS analysis revealed that M700F002 was also present hidden under the M700F007 peak. Therefore, it can be assumed that M700F007 was actually formed in smaller amounts than 7.5% TAR. In the aniline-label experiments, no corresponding counterpart containing the aniline-moiety of the molecule was found. No peak ever exceeded 1.4% TAR in the water phase. In sediment, fluxapyroxad reached its maximum in both systems and with both labels already after 43 days and declined again towards the last sampling day. A few more degradation products were formed in sediment compared to the dark incubations, however, none of them ever exceeding 2.5% TAR at any sampling time.

It can be concluded that under photolytic conditions, fluxapyroxad tends to split into a pyrazole moiety and an aniline moiety, which undergo separate degradation pathways. The aniline moiety seems to be preferably bound to the humic structures in the sediment, whereas the pyrazole moieties (M700F001, M700F002, M700F007) are found in the water phase. The material balance in the test vessels ranged between 92.0% and 106.7% TAR.

Overall, fluxapyroxad was observed to undergo rapid partitioning from the water phase to sediment in both systems. Given the high levels of TAR and low levels of metabolites at study termination, further degradation of fluxapyroxad was limited.

Despite the rapid primary degradation, minimal mineralization was observed. A maximum of 1.1% TAR as CO_2 was observed in in the dark experiment by day 100 with a maximum of 2.8% TAR as CO_2 in the irradiated experiment by termination on day 57.

Kinetic analysis and calculations of DT_{50} and DT_{90} values was performed following the recommendations of the FOCUS Kinetics workgroup. The analysis was done by non-linear regression methods (Marquardt algorithm, ordinary least squares optimization). Kinetic evaluation for the dark study was only performed for the parent substance (no metabolite was formed), considering the different levels proposed by the FOCUS kinetics guidance. The analysis at P-I level (one-compartmental approach) was done for degradation in the whole system as well as dissipation from the water phase and dissipation in the sediment phase of the test systems. At the P-II level (two-compartmental approach: water and sediment), the kinetic analysis considered the degradation in water and sediment and the partitioning between both phases.

The kinetic evaluation regarding the parent compound is similar for the irradiated study as for the dark study. Furthermore, metabolite M700F001 was formed in the irradiated study from the

pyrazole label in the water compartment of both test systems. For metabolite M700F001, a pronounced decline phase in the water/sediment systems was not visible, therefore, an analysis at M-I level (decline of metabolite in whole system, water, or sediment) was not performed. An analysis at M-I level for estimation of the degradation rates in the whole water-sediment system, however, was performed. Data for parent and metabolite were used to estimate degradation rates and the formation fraction from parent to metabolite. For each data set, the kinetic models proposed by the FOCUS Kinetics guidance document were tested in order to identify the best-fit model. The selected best-fit models and the corresponding results of the DT_{50}/DT_{90} at 20°C calculations for fluxapyroxad are listed in the table below. For the purpose of classification, DT_{50} values have been converted to 12°C (following ECHA guidance) to reflect an environmentally relevant temperature.

Table 42: Summary of the selected best-fit models and endpoints of the kinetic evaluation for Fluxapyroxad (BAS 700 F) under dark and irradiated conditions

Compartment	System	Kinetics	DT_{50} [d] max. 20°C in dark and 26°C in light experiments	DT_{50} [d] 12°C	DT_{90} [d] max. 20°C in dark and 26°C in light experiments	chi^2
Dark experiment						
Whole system	Berghäuser Altrhein	HS	> 1000	>1896	> 1000	0.8
	Ranschgraben	SFO	694	1316	> 1000	0.8
Water	Berghäuser Altrhein	FOMC	3.4	6.4	88	1.4
	Ranschgraben	FOMC	5.1	9.7	264	2.5
Sediment	Berghäuser Altrhein	n.c.	n.c.	n.c.	n.c.	n.c.
	Ranschgraben	n.c.	n.c.	n.c.	n.c.	n.c.
Irradiated experiment						
Whole system	Berghäuser Altrhein	SFO	145	444	482	1.1
	Ranschgraben	SFO	116	355	387	0.9
Water	Berghäuser Altrhein	DFOP	3.4	10.4	56	3.9
	Ranschgraben	DFOP	7.0	21.5	56	3.5
Sediment	Berghäuser Altrhein	SFO	173	530	576	8.8
	Ranschgraben	SFO	119	364	394	11.5

n.c. could not be calculated
HS Hockey Stick
SFO Single First Order
FOMC First Order Multi-Compartment
DFOP Double First Order in Parallel

The study demonstrates 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. A light increase in degradation was observed under irradiation.

A difference was also noted in the metabolic pattern. Under dark conditions, metabolite M700F002 was formed only in low amounts. Irradiation led to formation of metabolites

M700F001 and M700F007 in the water phase, and the formation of bound residues in the sediment was enhanced.

The DT_{50} (12ºC) values in the water phase were ≤13 days reflecting dissipation to the sediment phase. In the sediment, DT_{50} (12ºC) values could only be obtained for the irradiated experiment and were determined to be 225 to 328 days. The whole system degradation DT_{50} (12ºC) values were 220 to >1896 days.

Overall, the study degradation information 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, Fluxapyroxad is considered not rapidly degradable for the purpose of classification and labelling.

11.1.4.3.2 Soil degradation

Various soil fate studies are presented in the DAR. These do not impact the environmental classification and are not included in this report.

11.1.4.4 Photochemical degradation

11.1.4.4.1 Soil photolysis

An aquatic photolysis study is available taking precedent over soil photolysis data for classification purposes. Therefore soil photolysis data presented in the DAR are not included in this report.

11.1.4.4.2 Aqueous photolysis

STUDY 1

Author(s)	Hassink J.
Year:	2009(b)
Title:	Aqueous photolysis of BAS 700 F
Test Guidelines:	According to FAO Revised Guidelines on Environmental Criteria for the Registration of Pesticides Revision 3 (28 August 1993); EEC 94/37; EEC 91/414; EPA 161-2; JMAFF No 12 Nosan No 8147; SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995); OECD Guideline Proposal - Phototransformation of Chemicals in Water - Direct Photolysis (December 2007)
Deviations from Guideline:	No
GLP Compliance:	Yes

Photolysis of fluxapyroxad in sterile aqueous buffer at pH 7 was investigated with [pyrazole-4-^{14}C]-labelled and [anilin-U-^{14}C]-labelled fluxapyroxad over a testing period of 15 days with continuous irradiation. The test solutions with a concentration of 1 mg/l fluxapyroxad were exposed to a Xenon arc lamp (wavelength > 290 nm) continuously for 15 days in glass reaction vessels with special quartz glass covers. The temperature was kept constant at 22°C. The headspace of the vessel was continuously flushed with air and volatiles were collected in a series of trapping solutions (ethylene glycol, H_2SO_4 and NaOH). Samples were taken at 0, 2, 6, 9, 12 and 15 days after treatment. Dark

control samples were incubated under the same conditions except for irradiation. For the photolysis and the dark control experiment the recovery was in the range of 92-108 % TAR.

In the photolysis experiment with [pyrazole-4-^{14}C]-labelled fluxapyroxad the concentration of the test item ranged between 93 % TAR and 100 % TAR with 98 % TAR detected at the end of the study. In the photolysis experiment with [anilin-U-^{14}C]-labelled fluxapyroxad the concentration values for the test item were 97-108 % TAR, with 100 % TAR at the end of the study. The results for dark control samples were similar.

It was demonstrated that fluxapyroxad was stable in water at pH 7 with and without the influence of light. No degradation half-life and no quantum yield were calculated.

STUDY 2

In the irradiated water/sediment study [Ebert (2009)] the metabolite M700F007 was observed in the water phase reaching a maximum of 7.5% TAR at one sampling time in one of the two water/sediment systems. Due to the chemical structure of this metabolite (pyrazole carboxamide), hydrolytic or photolytic cleavage of the carboxamide group seemed possible. Therefore, a separate aquatic photolysis study was conducted with M700F007, in order to check the photolytic stability of this compound.

Author(s)	Hassink J.
Year:	2009(c)
Title:	Photolysis of M700F007 (metabolite of BAS 700 F) in sterile natural water
Test Guidelines:	(According to FAO Revised Guidelines on Environmental Criteria for the Registration of Pesticides Revision 3 (28 August 1993); JMAFF No 12 Nosan No 8147; EEC 91/414; EEC 94/37)
Deviations from Guideline:	No
GLP Compliance:	Yes

Photolysis of unlabelled M700F007 in sterile natural water was investigated over a testing period of 15 days with continuous irradiation. The test solutions with a concentration of 0.8 g/L M700F007 were placed under a SUNTEST system and continuously exposed to a Xenon arc lamp emitting a light spectrum similar to sunlight (>290 nm) at an intensity of about 3 mW/cm^2 simulating a clear summer day at Limburgerhof, south Germany (about 48° N). Glass reaction vessels with special quartz glass covers were used for the experiment. The test vessels were connected to an air flowthrough system. The head space of the vessel was continuously flushed with CO_2-free, moistened air. The test temperature was kept constant at 22°C. Samples were taken at 0, 1, 2, 6, 9, 13 and 15 days after treatment. Dark control samples were incubated under the same conditions except for irradiation. Recoveries of 91-104% of the applied test item were obtained in the irradiated test systems during the testing period of 15 days, 99-100 % were obtained for the dark control samples.

M700F007 was stable in sterile natural pond water with and without the influence of light, representing 104.2 % (photolysis) and 99.6 % (dark control) at the end of the 15-day incubation period. Hence, no half-lives or DT_{90} values were calculated.

It was demonstrated that M700F007 is stable in sterile natural pond water at pH 8 and 22±1°C, with and without influence of light.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant for this substance.

11.3 Environmental fate and other relevant information

11.3.1 Adsorption

Author(s)	Hassink J., Stephan A.
Year:	2009(a)
Title:	Determination of the adsorption/desorption behaviour of BAS 700 F on different soils
Test Guidelines:	(According to OECD 106; EPA 163-1; EPA 835.1230)
Deviations from Guideline:	No
GLP Compliance:	Yes

In laboratory batch experiments the adsorption / desorption behaviour of radiolabelled fluxapyroxad was investigated on five European and two North American soils and one Japanese soil. The eight soils covered a range of pH ($CaCl_2$) from 5.2 to 7.6, a range of organic carbon content from 0.41% to 3.84% and 5 different USDA textural classes: 3 sandy loam, 2 silt loam, 1 loamy sand, 1 silty clay loam and 1 sand. The soils used were sieved to a particle size <2 mm. The soils were air-dried at ambient temperature until a constant weight was reached.

For the determination of the adsorption isotherm, five different concentrations (nominal 0.01, 0.05, 0.1, 0.5 and 1 mg/l) of the test item in 0.01 M $CaCl_2$ solutions were used. The ratio of soil versus test solution was 1/1, and the measurements were performed at the adsorption equilibrium time of 48 hours for the eight soils. The desorption part was carried out in two steps with the soil residue remaining from the adsorption isotherm determination, respectively from the first desorption step, by adding 0.01 M $CaCl_2$ solution without test items. The following adsorption parameters were measured for the test item fluxapyroxad in each soil: distribution coefficients K_d and K_{OC} at five concentration levels, the Freundlich adsorption coefficient $K_{F,}$ the Freundlich exponent 1/n, and the corresponding K_{FOC} values.

From the Freundlich adsorption isotherms, K_F values (adsorption coefficients) in the range from 2.5 to 17.9 ml/g. The lowest value was found with California soil and the highest value with Nierswalde soil. The K_{FOC} values ranged from 320 mL/g (La Gironda soil) to 1101 mL/g (Nierswalde soil). Ranging from 1/n = 0.875 to 0.945, the Freundlich adsorption exponent indicated in most cases a non-linearity of the adsorption with the concentration.

The Freundlich desorption coefficient K_{FdesI} of the first desorption step covered a range from 5.4 to 51.9 mL/g for the eight soils. The lowest value was found with California soil and the highest value with Nierswalde soil. The $K_{FOCdesI}$ values ranged from 963 mL/g (La Gironda soil) to 6334 mL/g (LUFA 2.1). Ranging from 1/n = 1.005 to 1.444, the Freundlich desorption I exponent is above 1 in any cases. The Freundlich desorption coefficient K_{FdesII} of the second desorption step covered a range from 4.1 to 28.2 mL/g for the eight soils. The lowest value was found with California soil and the highest value with Nierswalde soil. The $K_{FOCdesII}$ values ranged from 486 mL/g (La Gironda soil) to 1969 mL/g (LUFA 2.1),. Ranging from 1/n = 0.869 to 1.066, the Freundlich desorption II exponent is again in a range indicating non-linearity of desorption.

The compound showed a high adsorptive behaviour since the Freundlich adsorption coefficients K_F covered a range from 2.5 to 17.9 mL/g for the eight soils. The K_{FOC} values ranged from 320 mL/g to 1101 mL/g. Desorption values were generally higher than adsorption equivalents indicating irreversible adsorption.

11.4 Bioaccumulation

Table 43: Summary of relevant information on bioaccumulation of Fluxapyroxad

Method	Species	Exposure system	Results	Reference
Partition coefficient n-octanol/water OECD 117	Not applicable	Not applicable	Log P_{ow} at 20°C: 3.08 (deionized water) 3.09 at pH 4 3.13 at pH 7 3.09 at pH 9	Wilfinger, 2008
Bioaccumulation (BCF) study in fish OECD 305 EPA 165-4; EPA 850.1730; GLP	*Lepomis macrochirus* (Bluegill sunfish)	28 d exposure, 16 d depuration	Bioconcentration factor: Whole fish BCF steady state lipid normalised to 5% lipid: 46 to 47 l/kg (parent) Whole fish BCF steady state lipid normalised to 5% lipid: 110-119 l/kg (TRR) Clearance time: DT_{50} (depuration half-life) = 0.74 days DT_{90} (elimination) = 2.45 days	Anonymous (2009w)

11.4.1 Estimated bioaccumulation

Not available.

11.4.2 Measured partition coefficient and bioaccumulation test data

The measured log Pow for Fluxapyroxad (99.3% purity) is 3.13 at pH 7 and 20ºC.

A bioconcentration factor (BCF) study with Bluegill sunfish has been performed and resulted in the following lipid normalised (5%) BCFs:

- steady state BCF 46-47 (parent)
- steady state BCF (total radioactive residues) 110-119
- kinetic BCF 114-121 (growth corrected)

Rapid elimination of the test item was observed with a depuration half-life of <1 day and a DT_{90} of 2.41 to 2.45 days. A study summary is provided below (Anonymous (2009w)).

The bioaccumulation and metabolism of Fluxapyroxad was investigated using ^{14}C-fluxapyroxad (purity 97.9%) and *Lepomis macrochirus* (Bluegill sunfish) in a dynamic flow-through system. Separate groups of fish were exposed to the test item at target concentrations of 1.0 µg/l and 10.0 µg/l achieved by a continuous dilution of an aqueous stock solution for a 28-day uptake phase. Subsequently fish were exposed to a continuous flow of only dilution water for 16 days (depuration phase). A control experiment with fish exposed to diluent water only was maintained in parallel.The test design and conditions were as follows:

Test design:	Flow through system (28 d uptake, 16 d depuration); two test item concentrations plus a dilution water control, 90 fish per group. Fish maintained in glass aquaria. The test solution flowed continuously from the mixing vessels into the test aquaria. For depuration fish were transferred to glass aquaria containing dilution water only. Fish were sampled (5/group) on days 1, 2, 4, 7, 14, 21, 24, and 28 of the uptake period, and on days 1, 2, 4, 8, and 16 of the depuration period. The fish taken from the control group were further split into a sample of two fish for blank correction and into a sample of three fish for lipid determination. Additional 10 fish per group were sampled on day 14 and 28 of the uptake period for quantification of metabolites. After sampling, fish were sacrificed, blotted dry and the body length and weight were determined. Each fish was divided into 4 samples (filet = edible part, head, organs and carcass (all inedible parts). Water samples (2 x 10 mL/group) were taken on days -1, 0, 1, 2, 4, 7, 14, 21, 24, and 28 of the uptake period, and on days 1 and 2 of the depuration period. Additional samples of 1 L test solution per group were sampled on day 14 and 28 of the uptake period. Daily assessment of mortality, water temperature, flow meter and function of pumps were made.
Test conditions:	Test vessels: glass aquaria with silicon rubber seals (80 x 35 x 55 cm), water volume: approx. 100 L; flow-through system; Dilution water: non-chlorinated tap water (diluted with deionized water); temperature 23°C ± 2°C; pH 7.5 - 7.6; oxygen content 7.6 - 8.4 µg/L (> 60% of the saturation concentration); total hardness: 102 - 104 mg $CaCO_3$/L. Lightning: fluorescent tubes at room ceiling; photoperiod: 16 hours light : 8 hours dark; flow rates: 20.8 L/hour/test vessel (dilution water) during uptake and depuration, 20.8 mL/hour/treatment group (stock solution). Feeding: commercial fish diet (Ecostart 17) and on workdays frozen brine shrimp (*Artemia*).

The test item concentration in water was analysed by Liquid Scintillation Counting (LSC) and were within ± 20% of the nominal concentration for both dose groups. Mean measured concentrations of the test item (based on total radioactivity) during the uptake phase were 1.03 µg/L and 10.74 µg/L for the low and high test concentration group, respectively. After 2 days of depuration radioactivity levels in water were below 1% of the nominal exposure and reached background level after 4 days.

HPLC analysis of the tank water sampled at Day 14 and 28 of the uptake phase showed that the only compound in water was unchanged fluxapyroxad, representing between 90% and 99% of the radioactivity.

Radioactivity levels in fish were determined by LSC for edible and inedible tissue, and calculated for whole fish. The respective values at the plateau level around Day 7 to Day 28 of the uptake phase were 0.026 µg/g, 0.105 µg/g and 0.089 µg/g, for the low dose level, and 0.264 µg/g, 1.195 µg/g and 0.996 µg/g for the high dose level.

Rapid elimination of radioactivity from fish was observed since after four to eight days of depuration residual radioactivity was below 5% of the plateau level.

The BCF values at steady-state (BCF_{ss}) based on TRR were calculated using the One Fish Compartment Model and linear regression analysis using Model Maker software. Kinetic BCF parameters were based on non-linear regression analysis using Model Maker Software.

At the time of the study, BCFs were calculated as lipid normalised whole fish BCFs considering a

standard lipid content of 6% and a study lipid content of 3.9% (mean of uptake day 7-28 lipid measurements). These were 132 to 143 l/kg and used in the assessment under Dir. 91/414/EEC. For the purpose of CLH, lipid normalisation to 5% lipid content is standard reflecting updates to the OECD TG 305. Lipid normalised (5%) kinetic and steady-state BCFs are included in Table 44 based on a lipid content of 3.9%. The is considered more conservative than a lipid normalisation based on a lipid content of 6.2% which was observed at the end of the depuration phase.

Table 44: Bioconcentration factors (BCF) and relevant kinetic parameters

Parameter	BCFs	
	Group 1 (1.0 µg/l)	Group 2 (10 µg/l)
	Whole fish	Whole fish
Uptake rate constant k_1 [$days^{-1}$]	82.9	89.0
Depuration rat constant k_2 [$days^{-1}$]	0.94	0.95
BCF_{KL} (= k_1/k_2) [l/kg] (growth corrected and lipid normalised)	114	121
BCF_{SSL} lipid normalised to 5% [l/kg] (based on total radioactive residues)	110	119
BCF_{SSL} lipid normalised to 5% [l/kg] (based on parent)	46	47
Depuration half-life DT_{50} [days]	0.74	0.73
DT_{90} [days]	2.45	2.41

BCF_{KL} = Kinetic BCF lipid normalised to %% lipid content
BCF_{SSL} = BCF at steady state lipid normalised to 5% lipid content
DT_{90} = Time to reach 90% depuration

The nature of radioactive residues in fish tissues at steady-state (Uptake day 14 and 28) was investigated after extraction with acetonitrile. HPLC analysis of the extracts showed considerable amounts of the unchanged parent compound and one metabolite M700F005. Quantitatively, fluxapyroxad accounted for 32% to 47% TRR in whole fish, whereas metabolite M700F005 represented from 16% to 23% TRR.

In addition, up to three minor metabolites were identified with all of them accounting for less than 10% of the total radioactivity in whole fish.

Reflecting residual amounts of unchanged parent compound accounting for 32% to 47% of the TRR in whole fish, whole fish BCFs were calculated based on parent.

The study demonstrates Fluxapyroxad underdoes some metabolic transformation to metabolites of higher polarity after uptake in Bluegill sunfish. Accumulation and elimination of fluxapyroxad in Bluegill sunfish were both rapid and followed first order kinetics. The calculated BCFs and rapid elimination indicate low bioconcentration potential. All experimental BCF values are below the BCF trigger of ≥ 500 l/kg intended to identify substances with a potential to bioaccumulate.

11.5 Acute aquatic hazard

Studies reviewed under Directive 91/414/EEC and considered reliable are summarised in the DAR. A summary of available valid information on the acute aquatic ecotoxicity of Fluxapyroxad is presented in Table 45.

The lowest endpoint is highlighted in bold for each trophic group. Additional ecotoxicity data (fish, invertebrates and algae) is available for three metabolites (MF700F001, MF700F002 and MF700F007) in the DAR which indicates these are less toxic than Fluxapyroxad.

Table 45: Summary of relevant information on acute aquatic toxicity

Species	Time-scale, guideline and GLP status	Substance	Endpoint result	Reference
Fish				
Oncorhynchus mykiss Rainbow trout	Acute, 96 h static OECD 203 EPA 850.1075 GLP	Fluxapyroxad as BAS 700 F (purity 99.5%)	LC_{50} = 0.546 mg a.s./l (n verified)	Anonymous (2007)
Lepomis macrochirus Bluegill sunfish	Acute, 96 h static OECD 203 EPA 850.1075 GLP	Fluxapyroxad as BAS 700 F (purity 99.7%)	LC_{50} =1.15 mg a.s./l (mm)	Anonymous (2008i)
Pimephales promelas Fathead minnow	Acute, 96 h static OECD 203 EPA 850.1075 GLP	Fluxapyroxad as BAS 700 F (purity 99.4%)	LC_{50} =0.466 mg a.s./l (mm)	Anonymous (2009x)
Cyprinus carpio Common carp	Acute, 96 h Semi-static JMAFF No 12 Nosan No 8147 GLP: yes	Fluxapyroxad as BAS 700 F (purity 99.4%)	**LC_{50} = 0.290 mg a.s./l** (mm)	Anonymous (2008j)
Cyprinodon variegatus Sheepshead minnow	Acute, 96 h static EPA 850.1075 GLP: yes	Fluxapyroxad as BAS 700 F (purity 99.5%)	LC_{50} = 1.30 mg a.s./l (mm)	Anonymous (2009y)
Aquatic invertebrates				
Daphnia magna	Acute, 48 h static OECD 202; EPA 850.1010 GLP: yes	Fluxapyroxad as BAS 700 F (purity 99.7%)	EC_{50} = 6.78 mg a.s./l (mm)	Janson, 2009a
Americamysis bahia	Acute, 96 h static EPA 850.1035; ASTM E 729 GLP: yes	Fluxapyroxad as BAS 700 F (purity 99.4%)	LC_{50} = 6.1 mg a.s./l (48 h) LC_{50} = 3.6 mg a.s./l (96 h) (mm)	Gallagher *et al*, 2009a
Crassostrea virginica	Acute, 96 h, flow-through, ASTM E729, EPA 850.1025	Fluxapyroxad as BAS 700 F (purity 99.4%)	**$EC_{50\ shell\ deposition}$ 1.1 mg a.s./l** LC_{50} >2.8 mg.a.s./l (mm)	Gallagher *et al*, 2009b
Algae and aquatic plants				
Pseudokirchneriella subcapitata	72 h , static OECD 201 GLP: yes	Fluxapyroxad as BAS 700 F (purity 99.5%)	**E_rC_{50} = 0.700 mg a.s./l** (n verified)	Hoffmann, 2008a, 2009a, 2010

Species	Time-scale, guideline and GLP status	Substance	Endpoint result	Reference
Anabaena flos-aquae	72 h , static OECD 201; EPA 850.5400 GLP: yes	Fluxapyroxad as BAS 700 F (purity 99.5%)	E_rC_{50} = 2.61 mg a.s./l (mm)	Hoffmann, 2009b
Navicula pelliculosa	72 h , static OECD 201; EPA 850.5400 GLP: yes	Fluxapyroxad as BAS 700 F (purity 99.5%)	E_rC_{50} > 3.42 mg a.s./l (mm)	Hoffmann, 2009c
Skeletonema costatum	72 h , static OECD 201; EPA 850.5400 GLP: yes	Fluxapyroxad as BAS 700 F (purity 99.5%)	E_rC_{50} = 5.88 mg a.s./l (mm)	Hoffmann, 2009d
Lemna gibba	7 d, static OECD 221; ASTM E 1415-91; EPA 850.4400 GLP: yes	Fluxapyroxad as BAS 700 F (purity 99.5%)	$E_rC_{50(\text{frond number})}$ > 3.43 mg a.s./l $E_rC_{50(\text{dry weight})}$ > 3.43 mg a.s./l (mm)	Hoffmann, 2009f

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

11.5.1 Acute (short-term) toxicity to fish

Five acute (short-term) fish studies are available with the active substance Fluxapyroxad (tested as BAS 700 F) which are summarised in the DAR (2011) and assessed in the EFSA conclusion (EFSA Journal 2012; 10(1):2522).

The lowest acute fish toxicity endpoint is derived from a study on common carp –. The studies were all performed to relevant guidelines under GLP and considered valid. Further details are below.

STUDY 1
Anonymous (2008j)

Executive Summary

In a static acute toxicity laboratory study, common carp (*Cyprinus carpio*) were exposed to 0.18, 0.24, 0.32, 0.42 and 0.56 mg/l fluxapyroxad (nominal) in groups of 10 animals in glass aquaria containing 50 litres of water. Fish were observed for survival and symptoms of toxicity 24, 48, 72 and 96 h after start of exposure.

The results are based on the mean measured concentrations. After 96 h of exposure no mortality and no toxic effects were observed in the control, the solvent control and at the lowest tested concentration of 0.168 mg/l fluxapyroxad (mean measured), whereas 100% mortality was observed at the two highest concentrations of 0.413 and 0.545 mg/l. At test item concentrations of 0.244 mg/l and 0.307 mg/l 10% and 60% mortality occurred. Sub-lethal effects were found at 0.244 mg/l (lethargy) and 0.307 mg/l (lethargy and lying laterally).

In a static acute toxicity study with common carp the LC_{50} (96 h) for fluxapyroxad was determined to be 0.29 mg/l based on mean measured concentrations.

I. MATERIAL AND METHODS

Test item:	Fluxapyroxad (Reg. No. 5 094 351), batch no. COD-001026, purity: 99.4%
Test species:	Common carp (*Cyprinus carpio*), mean body length 5.1 cm (4.9 - 5.5 cm), mean body weight 1.7 g (1.4 - 2.0 g).
Test design:	Semi-static system (96 hours); water renewal at 24-hour intervals; 10 fish per aquarium and per concentration; assessment of mortality and symptoms of toxicity 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, sub-lethal effects.
Test concentrations:	Control, solvent control, 0.18, 0.24, 0.32, 0.42 and 0.56 mg/l fluxapyroxad (nominal).
Test conditions:	Glass aquaria, test volume 50 L, dechlorinated, filtered tap water; temperature: 21.2 °C - 22.3 °C; pH 7.3 - 8.0; oxygen content: 6.5 mg/l - 8.5 mg/l; total hardness: about 43 mg $CaCO_3$/L; photoperiod 16 h light: 8 h dark; no aeration, no feeding.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.
Statistics:	Descriptive statistics; probit analysis for calculation of LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: At the start of exposure and after water exchange at 24, 48 and 72 h of exposure, the analytically detected concentrations of the test item ranged from 86% to 117% of the nominal values. At the end of exposure and before water exchange at 24, 48 and 72 h of exposure the measured values were between 88% and 117% of nominal. The biological results are based on mean measured concentrations.

Biological results: After 96 h of exposure no mortality and no toxic effects were observed in the control, the solvent control and at the lowest tested concentration of 0.168 mg fluxapyroxad/L (mean measured), whereas 100% mortality was observed at the two highest concentrations of 0.413 and 0.545 mg/l. At test item concentrations of 0.244 mg/l and 0.307 mg/l 10% and 60% mortality occurred, respectively. Sub-lethal effects were found at 0.244 mg/l (lethargy) and 0.307 mg/l (lethargy and lying laterally). The results are summarised in Table .

Table 46: Acute toxicity (96 h) of fluxapyroxad to common carp (*Cyprinus carpio*)

Concentration [mg/l] nominal	**Control**	**Solvent Control**	**0.18**	**0.24**	**0.32**	**0.42**	**0.56**
Concentration [mg/l] mean measured	--	--	**0.168**	**0.244**	**0.307**	**0.413**	**0.545**
Mortality [%]	0	0	0	10	60	100	100
Symptoms *	none	none	none	le	le/l.l.	n.d.	n.d.
	Endpoints [mg/l fluxapyroxad] (mean measured)						
LC_{50} (96 h)	0.29 (95% confidence limits: 0.27 - 0.33)						
NOEC (96 h)	0.168						

* Symptoms: le = lethargy; l.l. = lying laterally
n.d. = not determined; all fish dead

III. CONCLUSION

In a static acute toxicity study with common carp the LC_{50} (96 h) for fluxapyroxad was determined to be 0.29 mg/l based on mean measured concentrations.

STUDY 2

Anonymous (2007)

Executive Summary

In a static freshwater acute toxicity laboratory study, juvenile rainbow trout were exposed to nominal concentrations of fluxapyroxad at 0, 0.05, 0.10, 0.22, 0.5 and 1.0 mg a.s./l. Treatment groups and control comprised two replicates, each containing ten fish. Observations for mortality and symptoms of toxicity were made 1, 6, 24, 48, 72 and 96 hours following the start of the study.

Analysis of the test media confirmed that the measured concentrations were within 20 % of the nominal, therefore nominal concentrations were used to determine the toxicological endpoints. After 96 hours, 100 % mortality was evident at 1.0 mg a.s./l, with 15 % mortality noted at 0.5 mg a.s./L. There were no deaths or signs of toxicity up to and including 0.22 mg a.s./l.

In a static acute toxicity study with rainbow trout the LC_{50} (96 h) for fluxapyroxad was determined to be 0.546 mg/l based on verified nominal concentrations.

I. MATERIAL AND METHODS

Test item:	Fluxapyroxad (Reg. No. 5 094 351), batch no. COD-000899, purity: 99.7% (tolerance ± 1%).
Test species:	Rainbow trout (*Oncorhynchus mykiss*), aged 5 months old, with mean body length of 5.4 cm (5.2 - 5.5 cm) and mean body weight of 1.80 g (1.34 - 2.33 g).
Test design:	Static, non-renewal system (96 hours) comprising two replicates per treatment group, each containing 10 fish per aquarium (loading 0.36 g fish/l). The experimental endpoints were mortality and symptoms of toxicity. Observations commenced 1, 6, 24, 48, 72 and 96 hours following the start of exposure.
Endpoints:	LC_{50} and sub-lethal effects.
Test concentrations:	Control (0), 0.05, 0.10, 0.22, 0.5 and 1.0 mg/l fluxapyroxad (nominal).
Test conditions:	Fish were housed in glass aquaria with stainless steel frames (60 x 35 x 40 cm) containing 50 litres of non-chlorinated, filtered tap water. Aquaria were maintained at a temperature of 12 to 13 °C, a photoperiod of 16 h light to 8 h dark and light intensity of 82 - 293 Lux. Fish were not feed for the duration of the study. Water physico-chemical properties were as follows: pH 7.9 - 8.3; oxygen content 7.1 - 10.5 mg/l; total hardness of approximately 100 mg $CaCO_3$/l and conductivity of 250 µS/cm^2.
Analytics:	Analytical verification of test item concentrations was conducted using HPLC with MS detection.
Statistics:	Probit analysis was used to calculate the LC_{50}.

II. RESULTS AND DISCUSSION

Analytical verification of test item concentration was conducted in each concentration at 0 and 96 hours. Measured concentrations for fluxapyroxad ranged from 87.4% to 101.6% of nominal at 0 hours and from 81.2% to 102.6% of nominal at 96 hours. The following biological results are based on nominal concentrations.

After 96 hours of exposure no mortality and toxic effects were observed in the control and at concentrations of up to and including 0.22 mg a.s/L. Total mortality was observed at the highest test item concentration of 1.0 mg a.s./l. At a concentration of 0.50 mg a.s./l, 15% mortality was observed after 96 hours and surviving fish exhibited sub-lethal effects (apathy). The results are summarised in Table 47.

Table 47: Acute toxicity (96 h) of fluxapyroxad to rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/l] nominal	**Control**	**0.05**	**0.10**	**0.22**	**0.50**	**1.0**
Mortality [%]	0	0	0	0	15	100
Symptoms	none	none	none	none	A	n.d.
Endpoints [mg/l fluxapyroxad] (nominal)						
LC_{50} (96 h)	0.546					

Symptoms: A = apathy
n.d. = not determined; all fish dead

III. CONCLUSION

In an acute toxicity study using rainbow trout, the LC_{50} (96 h) for fluxapyroxad was determined to be 0.546 mg a.s./L, based on verified nominal concentrations.

STUDY 3

Anonymous (2008i)

Executive Summary

In a 96 hour static freshwater acute toxicity study, juvenile Bluegill sunfish (*Lepomis macrochirus*) were exposed to 0, 6.25%. 12.5%, 25%, 50% and 100% of a saturated solution, corresponding to mean measured concentrations of 0, 0.279, 0.572, 1.21, 2.41 and 5.05 mg/l fluxapyroxad. Treatment groups and control comprised two replicates, containing 10 fish. Observations were made at 1, 6, 24, 48, 72 and 96 hours following the start of exposure.

The test concentrations were determined using HPLC and the experimental endpoints are based on the mean measured concentrations. After 96 hours, 100 % mortality was evident at 2.41 and 5.05 mg a.s./L. No mortality was observed in the control and concentrations up to and including 0.572 mg/l fluxapyroxad. At 1.21 mg/l fluxapyroxad, 70% mortality was observed and all surviving fish showed sub-lethal effects (apathy and tottering).

In a static acute toxicity study, the LC_{50} (96 h) of Bluegill sunfish exposed to fluxapyroxad was determined to be 1.15 mg a.s./L (based on mean measured concentrations).

I. MATERIAL AND METHODS

Test item:	Fluxapyroxad (Reg. No. 5 094 351), batch no. COD-000899, purity: 99.7% (tolerance ± 1%).
Test species:	Bluegill sunfish (*Lepomis macrochirus*), 7 months old with mean body length 4.4 cm (4.1 - 4.7 cm) and mean body weights of 0.99 g (0.79 - 1.27 g).
Test design:	Static non-renewal system (96 hours). Control and treatment groups comprised two replicates, containing ten fish per aquarium (loading 0.33 g fish/l). Experimental endpoints were mortality and symptoms of toxicity. Observations were made 1, 6, 24, 48, 72 and 96 hours following start of study.
Endpoints:	LC_{50} and sub-lethal effects.
Test concentrations:	Control (0%), 6.25%, 12.5%, 25%, 50% and 100% of a saturated solution, corresponding to mean measured concentrations of 0, 0.279, 0.572, 1.21, 2.41 and 5.05 mg/l fluxapyroxad.
Test conditions:	Animals were housed in glass aquaria with stainless steel frames (60 x 35 x 40 cm) containing 30 litres of non-chlorinated, filtered tap water. Test conditions were maintained at a temperature of 23 ± 1 °C, a photoperiod 16 h light: 8 h dark and light intensity of 100 to 490 Lux. The tanks were not aerated and fish were not fed for the duration of the study. The physico-chemical parameters of the water were as follows: pH 7.8 - 8.3, oxygen content 5.9 - 8.5 mg/l, Total hardness 100 mg $CaCO_3$/l and conductivity of 250 µS/cm^2.
Analytics:	Analytical verification of test item concentrations was conducted using reverse phase HPLC with MS detection.
Statistics:	Probit analysis was used to calculate the LC_{50}.

II. RESULTS AND DISCUSSION

Analytical verification of test item concentration was conducted in each concentration at test initiation and (with the exception of the highest test concentration) after 48 and 96 hours. The mean measured concentration of the test item in the saturated solution (highest test concentration) was 5.05 mg/l fluxapyroxad at test initiation. In the lower concentrations the analytically determined concentrations of the test substance were constant over all measurements in the range of ± 20% of the nominal concentrations (% saturated solution). The biological results are based on mean measured concentrations.

After 96 hours, 100% mortality was observed at 2.41 and 5.05 mg a.s./l. No mortality was observed in the control and at concentrations of up to and including 0.572 mg/l fluxapyroxad. At a concentration of 1.21 mg/l fluxapyroxad, 70% mortality was observed and all surviving fish showed sublethal effects (apathy and tottering). The results are summarised in Table 48.

Table 48: Acute toxicity (96 h) of fluxapyroxad to Bluegill sunfish (*Lepomis macrochirus*)

% saturated solution	**Control**	**6.25**	**12.5**	**25**	**50**	**100**
Concentration [mg/l] mean measured	**0**	**0.279**	**0.572**	**1.21**	**2.41**	**5.05**
Mortality [%]	0	0	0	70	100	100
Symptoms	none	none	none	A/T	n.d.	n.d.
Endpoints [mg/l fluxapyroxad] (mean measured)						
LC_{50} (96 h)	1.15					

Symptoms: A = apathy; T = tottering
n.d. = not determined; all fish dead

III. CONCLUSION

In a static acute toxicity study with Bluegill sunfish the LC_{50} (96 h) for fluxapyroxad was determined to be 1.15 mg/l based on mean measured concentrations.

STUDY 4
Anonymous (2009x)

Executive Summary

In a static freshwater acute toxicity laboratory study, juvenile fathead minnows (*Pimephales promelas*) were exposed to 0, 6.25%. 12.5%, 25%, 50% and 100% of a saturated solution, corresponding to mean measured concentrations of 0, 0.327, 0.664, 1.42, 2.75 and 5.91 mg/l fluxapyroxad. Control and treatments groups comprised two replicates, each containing ten fish. Observations of mortality and symptoms of toxicity were made 1, 6, 24, 48, 72 and 96 hours following study initiation.

Toxicity endpoints are based on the mean measured concentrations. After 96 hours of exposure 100% mortality was observed at concentrations of 0.664, 1.42, 2.75 and 5.91 mg/l. No mortality and/or toxic effects were observed in the control and at 0.327 mg/l fluxapyroxad.

In a static acute toxicity study with fathead minnow the LC_{50} (96 h) for fluxapyroxad was determined to be 0.466 mg/l (based on mean measured concentrations).

I. MATERIAL AND METHODS

Test item:	Fluxapyroxad (Reg. No. 5 094 351), batch no. COD-001026, purity: 99.4%.
Test species:	Fathead minnow (*Pimephales promelas*), approximately 4 months old, with mean body lengths of 3.0 cm (2.7 - 3.2 cm) and mean body weights of 0.20 g (0.15 - 0.26 g). Test organisms were raised in-house.
Test design:	The study was conducted in a static, non-renewal system (96 hours). Control and treatment groups comprise two replicates, each containing ten fish per aquarium (loading 0.07 g fish/l). Observations were made 1, 6, 24, 48, 72 and 96 hours following the start of exposure.
Endpoints:	LC_{50} and sub-lethal effects.
Test concentrations:	Control (0%), 6.25%, 12.5%, 25%, 50% and 100% of a saturated stock solution, corresponding to mean measured concentrations of 0, 0.327, 0.664, 1.42, 2.75 and 5.91 mg/l BAS 700.
Test conditions:	Animals were housed in glass aquaria with stainless steel frames (60 x 35 x 40 cm) containing 30 litres of non-chlorinated, filtered tap water. Study conditions were maintained at a temperature of 22 ± 1 °C, a photoperiod 16 h light: 8 h dark and light intensity of 100 to 490 Lux. Tanks were not aerated and fish were not fed for the duration of the study. The physico-chemical parameters of the water were as follows: pH 8.0 - 8.2; oxygen content: 7.7 to 8.5 mg/l; total hardness: about 100 mg $CaCO_3$/l; conductivity: approximately 250 µS/cm^2.
Analytics:	Analytical verification of test item concentrations was conducted using reverse phase HPLC with MS detection.
Statistics:	Probit analysis was used to calculate the LC_{50}.

II. RESULTS AND DISCUSSION

Analytical verification of test substance concentration was conducted in each treatment group at test initiation and (with the exception of the highest test concentration) after 48 hours of exposure and (with the exception of the three highest concentrations) after 96 hours. At 0 hours, the mean measured concentration of the test item in the saturated solution (highest test concentration) was 5.91 mg fluxapyroxad/L. In the lower concentrations the analytically determined concentrations of the test substance were constant over all measurements in the range of ±20% of the initially measured concentrations. The toxic endpoints are based on mean measured concentrations.

After 96 hours, 100% mortality was observed at concentrations of 0.664, 1.42, 2.75 and 5.91 mg a.s./l. No mortality and/or toxic effects were observed in the control or at 0.327 mg/l fluxapyroxad. The results are summarised in Table 49.

Table 49: Acute toxicity (96 h) of fluxapyroxad to fathead minnow (*Pimephales promelas*)

% saturated solution	**Control**	**6.25**	**12.5**	**25**	**50**	**100**
Concentration [mg/l] mean measured	**0**	**0.327**	**0.664**	**1.42**	**2.75**	**5.91**
Mortality [%]	0	0	100	100	100	100
Symptoms	none	none	n.d.	n.d.	n.d.	n.d.
Endpoints [mg/l fluxapyroxad] (mean measured)						
LC_{50} (96 h)	0.466					

n.d. = not determined; all fish dead

III. CONCLUSION

In a static acute toxicity study with fathead minnow the LC_{50} (96 h) for fluxapyroxad was determined to be 0.466 mg/l based on mean measured concentrations.

STUDY 5

Anonymous (2009y)

Executive Summary

In a static marine acute toxicity study, sheepshead minnow were exposed to nominal concentrations of 0, 0.19, 0.38, 0.75, 1.5 and 3.0 mg/l fluxapyroxad, for a duration of 96 hours. Control, solvent control and treatment groups comprised two replicates, each containing 10 fish. Observations of mortality and symptoms of toxicity were made 3, 24, 48, 72 and 96 hours following study commencement.

After 96 hours, 100% mortality was observed at 2.8 mg a.s./l. No mortality or toxic effects were observed in the control and at concentrations of up to and including 0.37 mg/l fluxapyroxad. No mortalities occurred in the 0.72 mg/l treatment group, but some of the fish were lethargic at test termination. At a concentration of 1.5 mg/l fluxapyroxad 70% mortality was observed and some of the surviving fish exhibited lethargy.

In a static marine acute toxicity study the LC_{50} (96 h) of fluxapyroxad to sheepshead minnow was 1.3 mg/l (based on mean measured concentrations).

I. MATERIAL AND METHODS

Test item:	Fluxapyroxad (Reg. No. 5 094 351), batch no. COD-001026, purity: 99.4%.
Test species:	Sheepshead minnow (*Cyprinodon variegatus*), approximately 3 months old, with mean body lengths of 2.2 cm (1.7 - 2.6 cm) and mean body weights of 0.21 g (0.10 - 0.46 g).
Test design:	The study was conducted in a static marine system for 96 hours. Control and treatment groups comprised two replicates, each containing ten fish (loading 0.14 g fish/l). Observations of mortality and symptoms of toxicity were made 3, 24, 48, 72 and 96 hours following study commencement.
Endpoints:	LC_{50} and sub-lethal effects.
Test concentrations:	Control (dilution water), solvent control (0.1 ml/l dimethyl formamide), 0.19, 0.38, 0.75, 1.5 and 3.0 mg/l fluxapyroxad (nominal).
Test conditions:	Fish were housed in 25 litre stainless steel tanks, containing 15 L of filtered, diluted seawater. Study conditions were maintained at temperature of 21.3 to 22.2 °C, photoperiod of 16 h light : 8 h dark and light intensity of 768 Lux (test initiation). The tanks were not aerated and the fish were not fed for the duration of the study. The physico-chemical properties of the water were as follows: salinity: 20‰; pH 8.0 - 8.2; oxygen content: 5.0 - 8.5 mg/l.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC with UV detection.
Statistics:	Probit analysis was used to calculate the LC_{50}.

II. RESULTS AND DISCUSSION

Analytical verification of test item concentration was conducted in each concentration at test initiation, after 48 hours of exposure and (with the exception for the highest concentration) at test termination. Measured concentrations for fluxapyroxad ranged from 89.9% to 100% of nominal at test initiation and from 91.8% to 98.9% of nominal at test termination. The ecotoxicological endpoints are based on mean measured concentrations.

After 96 hours, 100% mortality was observed at the highest test item concentration of 2.8 mg a.s./l. No mortality or toxic effects were observed in the control or at concentrations up to and including 0.37 mg/l fluxapyroxad. No mortalities occurred at 0.72 mg a.s./l, albeit some fish exhibited lethargy at test termination. At a concentration of 1.5 mg/l fluxapyroxad, 70% mortality was observed and some of the surviving fish showed lethargy. The results are summarised in Table 50.

Table 50: Acute toxicity (96 h) of fluxapyroxad to Sheepshead minnow (*Cyprinodon variegatus*)

Concentration [mg/L] nominal	**Control**	**Solvent Control**	**0.19**	**0.38**	**0.75**	**1.5**	**3.0**
Concentration [mg/L] mean measured	**--**	**--**	**0.18**	**0.37**	**0.72**	**1.5**	**2.8**
Mortality [%]	0	0	0	0	0	70	100
Symptoms	none	none	none	none	C	C	n.d.
Endpoints [mg/L fluxapyroxad] (mean measured)							
LC_{50} (96 h)	1.3 (95% confidence limits: 0.72 - 2.8)						

Symptoms: C = lethargy.
n.d. = not determined; all fish dead.

III. CONCLUSION

In a static marine acute toxicity study, the LC_{50} (96 h) of fluxapyroxad in sheepshead minnow was 1.3 mg a.s./l (based on mean measured concentrations).

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Acute (short-term) studies on aquatic crustaceans are available for *Daphnia magna* and *Americamysis bahia* and the mollusc *Crassostrea virginica.* Summaries are presented in the DAR (2011) and assessed in the EFSA conclusion (EFSA Journal 2012; 10(1):2522).

The lowest endpoint relevant for classification was derived from the *Crassostrea virginica* study based on shell deposition.

STUDY 1

Author(s):	Gallagher S.P. et al.
Year:	2009(a)
Title:	BAS 700 F: A 96-hour static acute toxicity test with the saltwater mysid (*Americamysis bahia*)
Test Guidelines:	According to EPA 850.1035; ASTM E 729
Deviations from Guideline:	No
GLP Compliance:	Yes

Executive Summary

In a static acute toxicity laboratory study, saltwater mysids were exposed to 0.63, 1.3, 2.5, 5.0 and 10.0 mg/l Fluxapyroxad (tested as BAS 700 F) (nominal) in groups of 10 animals in glass beakers containing 1.5 litre water with 2 replicates per concentration. Saltwater mysids were observed for survival and symptoms of toxicity 4, 24, 48, 72 and 96 h after start of exposure.

The biological results are based on mean measured concentrations. After 96 hours of exposure no mortality and toxic effects were observed in the control, the solvent control and at the lowest tested concentration of 0.60 mg/l fluxapyroxad. Mortality rates of 10%, 10%, 55% and 100% after 96 h were observed in the 1.2, 2.3, 4.3 and 8.7 mg/l fluxapyroxad test item groups. At concentrations of 1.2 and above, signs of toxicity evident among mysids, included erratic swimming, surfacing and lethargy.

In a static acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (48 h and 96 h) for fluxapyroxad was determined to be 6.1 mg/l and 3.6 mg/l based on mean measured concentrations. The NOEC (96 h) was 0.60 lmg/l (mean measured).

I. MATERIAL AND METHODS

Test item:	Fluxapyroxad, Reg. No. 5 094 351), batch no. COD-001026, purity: 99.4%.
Test species:	Saltwater mysid (*Americamysis bahia*), juveniles, age: less than 24 h old; source: in-house cultures.

Test design:	Static system (96 h); 10 mysids per test chamber, 2 replicates per concentration; assessment of mortality and symptoms of toxicity approximately 4, 24, 48, 72 and 96 h after start of exposure.
Endpoints:	LC_{50} (96 h), NOEC, mortality and sub-lethal effects.
Test concentrations:	Control (dilution water), solvent control (0.1 ml/l dimethyl formamide), 0.63, 1.3, 2.5, 5.0 and 10.0 mg/l fluxapyroxad/ (nominal).
Test conditions:	2.0 L glass beakers, test volume 1.5 litre, filtered and diluted seawater, salinity: 20‰; temperature: 24.2 °C - 25.7 °C; pH 7.9 - 8.2; oxygen content: 4.6 mg/l - 7.6 mg/l; photoperiod 16 h light : 8 h dark; light intensity: 751 lux at test initiation; juvenile mysids were fed daily with live brine shrimps (*Artemia nauplii*).
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with variable wavelength detection.
Statistics:	Descriptive statistics; probit analysis for calculation of LC_{50} (96 h).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at test initiation and at test termination. Measured concentrations for fluxapyroxad ranged from 84.2% to 92.5% of nominal at test initiation and from 85.7% to 96.2% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure no mortality and toxic effects were observed in the control, the solvent control and at the lowest tested concentration of 0.60 mg/l fluxapyroxad. Mortality rates of 10%, 10%, 55% and 100% after 96 h were observed in the 1.2, 2.3, 4.3 and 8.7 mg/l fluxapyroxad test item groups. At concentrations of 1.2 and above, signs of toxicity evident among mysids, included erratic swimming, surfacing and lethargy. The results are summarised in Table.

Table 51: Acute toxicity (96 h) of fluxapyroxad to saltwater mysids (*Americamysis bahia*)

Concentration [mg/l] nominal	**Control**	**Solvent control**	**0.63**	**1.3**	**2.5**	**5.0**	**10.0**
Concentration [mg/l] mean measured	--	--	**0.60**	**1.2**	**2.3**	**4.3**	**8.7**
Mortality [%]	0	0	0	10	10	55	100
Symptoms *	none	none	none	C	C	C	n.d.
	Endpoints [mg/l fluxapyroxad] (mean measured)						
LC_{50} (48 h)	6.1 (95% confidence limits: 4.3 – 8.7)						
LC_{50} (96 h)	3.6 (95% confidence limits: 2.9 - 4.5)						
NOEC (96 h)	0.60						

* Symptoms: C = lethargy
n.d. = not determined; all mysids dead

III. CONCLUSION

In a static acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (48 h and 96 h) for fluxapyroxad was determined to be 6.1 mg/l and 3.6 mg/l based on mean measured concentrations. The NOEC (96 h) was 0.60 mg/l (mean measured).

STUDY 2

Author(s)	Gallagher S.P. et al.
Year:	2009(b)
Title:	BAS 700 F: A 96-hour shell deposition test with the eastern oyster (*Crassostrea virginica*)
Test Guidelines:	According to EPA 850.1025, ASTM E 729
Deviations from Guideline:	No
GLP Compliance:	Yes

Executive Summary

A study was conducted to determine the effects of fluxapyroxad on the shell deposition of Eastern oysters during a 96-hour exposure period under flow-through test conditions. The eastern oysters were exposed to 0, 0.078, 0.16, 0.31, 0.75, 1.3 and 2.5 mg/l (nominal). The control, solvent control and treatment groups comprised a single replicate, each containing 20 oysters. Observations of mortality and symptoms of toxicity were made 6.5, 24, 48, 72 and 96 hours following study initiation. Measurements of shell deposition for each oyster were made after 96 hours and were used to calculate the EC_{50}.

The toxicological endpoints are based on mean measured concentrations. After 96 hours of exposure, statistically significant inhibition of shell growth compared to the pooled control was observed at concentrations ≥0.77 mg/l. No mortalities and no other sub-lethal effects were observed in either of the control groups or the treatment groups.

In a study with eastern oysters (*Crassostrea virginica*) under flow-through test conditions, the $EC_{50\ shell\ growth}$ (96 h) was 1.1 mg/l based on mean measured concentrations.

I. MATERIAL AND METHODS

Test item:	Fluxapyroxad (Reg. No. 5 094 351), batch no. COD-001026, purity: 99.4%.
Test species:	Eastern oysters (*Crassostrea virginica*), juveniles with average length of 39.9 mm, ± 2.3 mm (range: 35.0 - 43.5 mm). Animals were sourced from Circle C Oyster Ranch, Ridge (Maryland), USA.
Test design:	The study was conducted in a flow-through system for a duration of 96 hours. Controls and treatments groups comprised a single replicate containing 20 oysters per glass aquarium. Assessment of mortality and symptoms of toxicity were made 6.5, 24, 48, 72 and 96 hours following study commencement and measurements of shell deposition were made 96 hours following study commencement.
Endpoints:	EC_{50} (96 h) and symptoms of toxicity.
Test concentrations:	Control (dilution water), solvent control (0.1 ml/l dimethyl formamide), 0.078, 0.16, 0.31, 0.75, 1.3 and 2.5 mg/l (nominal) equivalent to measured concentrations of 0, 0.082, 0.16, 0.32, 0.77, 1.1 and 2.2 mg a.s./l, respectively.
Test conditions:	The animals were housed in 54 litre glass aquaria for the duration of the study and contained 27 litres of filtered and diluted seawater. Environmental conditions were maintained at a temperature of 18.7 - 19.7 °C, a photoperiod 16 hours light: 8 hours dark; light intensity and 308 Lux at test initiation. Oysters were fed a suspension of marine microalgae at

	approximately 5.8 x 10^9 cells per oyster per day. The physico-chemical properties of the water were as follows: salinity of 20‰, pH 8.0 to 8.2 and dissolved oxygen content of 7.1 to 7.8 mg/l.
Analytics:	Analytical verification of test item concentrations was conducted using HPLC with UV detection.
Statistics:	Data were transformed prior to analysis. Differences in shell deposition were analysed using ANOVA and Dunnett‟s t-test ($\alpha \leq 0.05$) whereas linear interpolation was used to calculate the EC_{50} (96 h).

II. RESULTS AND DISCUSSION

Analytical measurements: Due to the interruption of stock solution flow to the 0.16 mg/l treatment group test chamber, the 0.16 mg/l test concentration was excluded from statistical analyses. Analytical verification of test item concentration was conducted in each concentration at test initiation and at test termination. Measured concentrations for fluxapyroxad ranged from 90.8% to 127.0% at test initiation and from 89.2 to 104.0% at test termination. The ecotoxicological endpoints are based on mean measured concentrations.

Biological results: After 96 hours, statistically significant inhibition (Dunnett t-test, $p \leq 0.05$) of shell growth compared to the pooled control was observed at concentrations in excess of 0.77 mg/l. No mortalities were evident in either the control or treatment groups. The results are summarised in Table 52.

Table 52: Acute toxicity (96 h) of fluxapyroxad to eastern oysters (*Crassostrea virginica*)

Concentration [mg/l] nominal	**Control**	**Solvent control**	**Pooled control**	**0.078**	**0.16**	**0.31**	**0.75**	**1.3**	**2.5**
Concentration [mg/l] mean measured	**--**	**--**	**--**	**0.082**	**0.16** [1)]	**0.32**	**0.77**	**1.1**	**2.2**
Shell growth inhibition after 96 h [%] [2)]	--	--	--	12	42	21	28*	66*	98*
Mortality after 96 h [%]	0	0	0	0	0	0	0	0	0
Symptoms	none	none	none	none	none	none	none	none	none
	Endpoints [mg/] (mean measured)								
$EC_{50\ shell\ growth}$ (96 h)	1.1 (95% confidence limits: 0.94 - 1.4)								

* Statistically significant difference compared to pooled control (Dunnett t-test, $p \leq 0.05$). Positive values indicate inhibition and negative values stimulation of shell growth.

1) Due to the interruption of stock solution flow to the 0.16 mg/L treatment group test chamber, the 0.16 mg/l test concentration was excluded from statistical analyses, i.e. calculation of the EC_{50} value.

2) Percent inhibition from pooled controls.

III. CONCLUSION

In a study with eastern oysters (*Crassostrea virginica*) under flow-through test conditions, the $EC_{50\ shell\ growth}$ (96 h) was established as 1.1 mg/l based on mean measured concentrations.

STUDY 3

Author(s)	Janson, G.
Year:	2009a
Title:	Acute 48 hour static toxicity study using *Daphnia magna* STRAUS
Test Guidelines:	According to EPA 850.1025, ASTM E 729
Deviations from Guideline:	No
GLP Compliance:	Yes

Executive Summary

In an acute, static toxicity study, *Daphnia* neonates were exposed to fluxapyroxad at nominal concentrations of 0, 0.62, 1.25, 2.5, 5.0 and 10 mg a.s./l for a duration of 48 hours. The control, solvent control and treatment groups comprised four replicates, each containing five daphnids. Observations of immobility were made 24 hours and 48 hours following study commencement.

The toxicological endpoints were presented as nominal and mean measured concentrations. After 48 hours, 100% immobility was observed at the highest test item concentration of 10 mg a.s./l (nominal); no immobility was observed in both control groups and at concentrations of up to and including 5.0 mg/l fluxapyroxad (nominal).

In a 48-hour static acute toxicity study, the EC_{50} of fluxapyroxad in *Daphnia magna* is 7.07 mg/l based on nominal concentrations and 6.78 mg/l based on mean measured concentrations.

I. MATERIAL AND METHODS

Test item:	Fluxapyroxad (Reg. No. 5 094 351), batch no. COD-000899, purity: 99.7% (tolerance ± 1%).
Test species:	Water flea (*Daphnia magna* STRAUS), neonates from in-house culture (originally obtained from Institut National de Recherche Chimique Appliquée, France); > 2 < 24 hours old at test initiation.
Test design:	The study was conducted in a static, non-renewal system for a duration of 48 hours and included a control, a solvent control and five test concentrations. The controls and treatment groups comprised four replicates. All replicates contained five daphnids. Immobility was assessed after 24 and 48 hours.
Endpoints:	EC_{50} based on immobility of daphnids.
Test concentrations:	Control, solvent control (DMSO/Cremophor; 1:1), 0.62, 1.25, 2.5, 5.0 and 10 mg/l fluxapyroxad (nominal).
Test conditions:	The study was conducted in glass vessels, containing 50 ml of dilution water "M4" (Elendt medium) for a period of 48 hours. The study conditions were maintained at a temperature of 20.2 to 20.7 °C, a light intensity of 275 - 614 Lux and a photoperiod 16 hours light: 8 hours dark. The vessels were not aerated and the animals were not fed for the duration of the study. The physico-chemical properties of the Elendt media were as follows: pH 7.94 - 8.03; oxygen content: 8.9 - 9.1 mg/l; total hardness: 2.47 mmol/L at test initiation, conductivity: 672 µS/cm at test initiation.
Analytics:	The test item concentrations were quantified by external calibration using HPLC with MS detection.

Statistics: No statistical analysis was conducted. The EC_{50} was calculated from the geometric mean of the NOEC and the LOEC.

II. RESULTS AND DISCUSSION

Analytical verification of test item concentration was conducted in each concentration at the beginning and at the end of the test. Measured values for fluxapyroxad ranged from 96.3% to 102.3% of nominal at test initiation and from 94.4% to 101.4% at test termination. The following toxicological endpoints are based on nominal and mean measured concentrations.

After 48 hours, 100% immobility was observed at the highest test item concentration of 10 mg a.s./l (nominal). No immobility was observed in both control groups and at concentrations of up to and including 5.0 mg/l fluxapyroxad (nominal). The results are summarised in Table 53.

Table 53: Immobility of *Daphnia magna* resulting from exposure to fluxapyroxad

Concentration [mg/l] nominal	**Control**	**Solvent control**	**0.62**	**1.25**	**2.5**	**5.0**	**10**
Concentration [mg/l] mean measured	**--**	**--**	**0.60**	**1.26**	**2.55**	**4.83**	**9.54**
Immobile (24 h) [%]	0	0	0	0	0	0	25
Immobile (48 h) [%]	0	0	0	0	0	0	100
Endpoints [mg/l fluxapyroxad]							
EC_{50} (48 h) nominal	7.07						
EC_{50} (48 h) mean measured	6.78						

III. CONCLUSION

In a 48-hour static acute toxicity study, the EC_{50} of fluxapyroxad in *Daphnia magna* was determined to be 7.07 mg/l based on nominal concentrations and 6.78 mg/l based on mean measured concentrations.

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

Four short-term studies on algae are available and one additional study with the aquatic macrophyte *Lemna gibba*. Summaries are included in the DAR (Draft Assessment Report 2011) and assessed in the EFSA conclusion (EFSA Journal 2012; 10(1):2522).

The lowest endpoint is derived from the study on green algae (*Pseudokirchneriella subcapitata).* Studies were performed to recent OECD/EPA guidelines under GLP and are seen as valid. Further details are below.

STUDY 1

Author(s)	Hoffmann F.
Year:	2008 (a)
Title:	Effect of BAS 700 F (Reg.No. 5094351) on the growth of the green alga *Pseudokirchneriella subcapitata*
Test Guidelines:	According to OECD 201
Deviations from Guideline:	No
GLP Compliance:	Yes

Author(s)	Hoffmann F.
Year:	2009(a)
Title:	Report amendment No. 1: Effect of BAS 700 F (Reg.No. 5094351) on the growth of the green alga *Pseudokirchneriella subcapitata*

Author(s)	Hoffmann F.
Year:	2010
Title:	Amendment No. 2 - Effect of BAS 700 F (Reg.No. 5094351) on the growth of the green alga *Pseudokirchneriella subcapitata*

Executive Summary

In a 96-hour static toxicity laboratory study, the effect of fluxapyroxad on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following concentrations were applied: 0.10, 0.15, 0.23, 0.34, 0.51, 0.76 and 1.14 mg/l fluxapyroxad (nominal). Assessment of growth was conducted 0 h, 24 h, 48 h, 72 h and 96 h after test initiation.

The biological results are based on nominal concentrations. No morphological effects on algae were observed in the control groups and at concentrations up to 0.76 mg/l fluxapyroxad. At the highest concentration of 1.14 mg a.s./l the cell density was too low for evaluation of cell morphology.

In a toxicity test with *Pseudokirchneriella subcapitata* the E_rC_{50} of fluxapyroxad was determined to be 0.70 mg/l (72 h) based on verified nominal concentrations. As an E_rC_{10} is available this is preferred to a NOEC given the statistical basis - the E_rC_{10} was 0.31 mg/l also based on verified nominal concentrations.

I. MATERIAL AND METHODS

Test item:	Fluxapyroxad (Reg. No. 5 094 351), batch no. COD-000899, purity: 99.7% (tolerance ± 1%).
Test species:	Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz), SAG 61.81; stock obtained from "Sammlung von Algenkulturen" Göttingen, Germany.
Test design:	Static system (96 hours); 7 test concentrations plus a control (without solvent) with 5 replicates for each and a solvent control with 10 replicates; daily assessment of growth.
Endpoints:	EC_{05}, EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 and 96 hours.
Test concentrations:	Control, solvent control (acetone), 0.10, 0.15, 0.23, 0.34, 0.51, 0.76 and 1.14 mg/l fluxapyroxad (nominal).
Test conditions:	100 ml Erlenmeyer flasks; nutrient solution according to OECD 201 (test volume: 60 mL); pH 8.1 at test initiation, pH 7.42 - 7.96 at test termination; temperature: 22 ± 1 °C; initial cell densities 6 x 10^3 cells/mL; continuous light at about 8000 lux; continuous shaking.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.
Statistics:	Descriptive statistics; probit analysis for yield data after 96 hours, logit analysis for growth rate data after 96 hours and log-log analysis for growth rate and yield data after 72 hours.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for fluxapyroxad ranged from 97.7% to 101.5% of nominal at test initiation and from 93.5% to 98.4% of nominal at test termination. The following biological results are based on nominal concentrations of the test item.

Biological results: No morphological effects on algae were observed in the control groups and at concentrations up to 0.76 mg/l fluxapyroxad. At the highest concentration of 1.14 mg a.s./l the cell density was too low for evaluation of cell morphology. The effects on algal growth are summarised in Table 54.

Table 54: Effect of fluxapyroxad on yield and growth rate of green alga *Pseudokirchneriella subcapitata* (72 h)

Concentration [mg/l] nominal	**Solvent control**	**Control**	**0.10**	**0.15**	**0.23**	**0.34**	**0.51**	**0.76**	**1.14**
Inhibition in 72 h (growth rate) [%] [1)]	--	-0.2	0.4	5.3	5.5	6.6	19.4	70.3	84.8
Inhibition in 96 h (growth rate) [%] [1)]	--	-0.3	-0.4	0.0	2.7	8.7	24.5	68.5	83.8
	Endpoints [mg/l fluxapyroxad] nominal								
E_rC_{50} (72 h)	0.70 (95% confidence limits: 0.69 - 0.72)								
E_rC_{10} (72 h)	0.31 (95% confidence limits: 0.30 - 0.33)								

[1)] Inhibition compared to the solvent control; negative values indicate stimulated growth.

III. CONCLUSION

In a toxicity test with *Pseudokirchneriella subcapitata* the 72 hour E_rC_{50} of fluxapyroxad was determined to be 0.70 mg/l based on verified nominal concentrations. As an E_rC_{10} is available this is preferred to a NOEC given the statistical basis - the E_rC_{10} was 0.31 mg/l also based on verified nominal concentrations.

STUDY 2

Author(s)	Hoffmann F.
Year:	2009(b)
Title:	Effect of BAS 700 F (Reg.No. 5094351) on the growth of the blue-green alga *Anabaena flos-aquae*
Test Guidelines:	According to OECD 201; EPA 850.5400
Deviations from Guideline:	No
GLP Compliance:	Yes

Executive Summary

In a 96-hour static toxicity laboratory study, the effect of fluxapyroxad on the growth of the blue-green alga *Anabaena flos-aquae* was investigated. The following geometric mean measured concentrations were applied: 0 (control), 0.24 mg/l (6.25% dilution), 0.48 mg/l (12.5% dilution),

0.93 mg/l (25% dilution), 1.88 mg/l (50% dilution) and 3.74 mg/l (100%, pure stock solution). Assessment of growth was conducted 0 h, 24 h, 48 h, 72 h and 96 h after test initiation.

The biological results are based on geometric mean measured concentrations. No morphological effects on algae were observed in the control group and at any of the test item concentrations tested.

In a toxicity test with *Anabaena flos-aquae* the E_rC_{50} of fluxapyroxad was determined to be 2.61 mg/l (72 h) and 3.53 mg/l (96 h) based on geometric mean measured concentrations. The 72 hour E_rC_{10} was 1.20 mg/l based on measured concentrations. The 96 hour E_rC_{10} was 1.72 mg/l based on measured concentrations.

I. MATERIAL AND METHODS

Test item:	Fluxapyroxad (Reg. No. 5 094 351), batch no. COD-000986, purity: 99.5% (tolerance ± 1.0%).
Test species:	Freshwater blue-green alga *Anabaena flos-aquae*, UTEX B 1444, stock obtained from "UTEX Culture collection of Algae", University of Texas at Austin, USA.
Test design:	Static system (96 hours); 5 test item concentrations with 5 replicates plus a control with 10 replicates; daily assessment of growth.
Endpoints:	EC_{05}, EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 and 96 hours.
Test concentrations:	Geometric mean measured concentrations: 0 (control), 0.24 mg/l (6.25% dilution), 0.48 mg/l (12.5% dilution), 0.93 mg/l (25% dilution), 1.88 mg/l (50% dilution) and 3.74 mg/l (100%, pure stock solution). The highest test concentration at test initiation reflects the maximum solubility of the test item under test conditions.
Test conditions:	100 mL Erlenmeyer flasks; nutrient solution according to OPPTS 850.5400, respectively, OECD 201 (test volume: 60 mL); pH 7.5 at test initiation, pH 7.57 - 7.87 at test termination; temperature: 24 ± 1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 4400 lux; continuous shaking.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.
Statistics:	Descriptive statistics; probit analysis for growth rate and yield data after 72 and 96 hours.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for set-ups with concentration levels of 6.25% up to 100% of a saturated stock solution of fluxapyroxad ranged from 0.250 mg/l to 3.749 mg/l at test initiation and from 0.238 mg/l to 3.740 mg/l at test termination. The biological results are based on geometric mean measured concentrations of the test item.

Biological results: No morphological effects on algae were observed in the control group and at any of the test item concentrations tested. The effects on algal growth are summarised in Table 55.

Table 55: Effect of fluxapyroxad on yield and growth rate of blue-green alga *Anabaena flos-aquae* (72 h and 96 h)

% saturated solution	**Control**	**6.25**	**12.5**	**25**	**50**	**100**
Concentration [mg/l] geom. mean measured	**Control**	**0.24**	**0.48**	**0.93**	**1.88**	**3.74**
Inhibition in 72 h (growth rate) [%] [1)]	--	-0.3	0.0	6.3	28.5	72.6
Inhibition in 96 h (growth rate) [%] [1)]	--	-0.7	-0.6	4.8	12.2	54.3
	Endpoints [mg fluxapyroxad/L] geom. mean measured					
E_rC_{50} (72 h)	2.61 (95% confidence limits: 2.47 - 2.77)					
E_rC_{10} (72 h)	1.20 (95% confidence limits: 1.04 - 1.33)					
E_rC_{50} (96 h)	3.53 (95% confidence limits: 3.41 - 3.68)					
E_rC_{10} (96 h)	1.72 (95% confidence limits: 1.57 - 1.86)					

[1)] Inhibition compared to control; negative values indicate stimulated growth

III. CONCLUSION

In a toxicity test with *Anabaena flos-aquae* the E_rC_{50} of fluxapyroxad was determined to be 2.61 mg/l (72 h) and 3.53 mg/l (96 h) based on geometric mean measured concentrations. The 72 hour E_rC_{10} was 1.20 mg/l based on measured concentrations. The 96 hour E_rC_{10} was 1.72 mg/l based on measured concentrations.

STUDY 3

Author(s)	Hoffmann F.
Year:	2009(c)
Title:	Effect of BAS 700 F (Reg.No. 5094351) on the growth of the fresh water diatom *Navicula pelliculosa*
Test Guidelines:	According to OECD 201; EPA 850.5400
Deviations from Guideline:	No
GLP Compliance:	Yes

Executive Summary

In a 96-hour static toxicity laboratory study, the effect of fluxapyroxad on the growth of the fresh water diatom *Navicula pelliculosa* was investigated. The following geometric mean measured concentrations were applied: 0 (control), 0.215 mg/l (6.25% dilution), 0.435 mg/l (12.5% dilution), 0.867 mg/l (25% dilution), 1.723 mg/l (50% dilution) and 3.418 mg/l (100%, pure stock solution). Assessment of growth was conducted 0 h, 24 h, 48 h, 72 h and 96 h after test initiation.

The biological results are based on geometric mean measured concentrations. No morphological effects on algae were observed in the control group and at any of the test item concentrations tested.

In a toxicity test with *Navicula pelliculosa* the E_rC_{50} of fluxapyroxad was determined to be 8.78 mg/l (72 h) and 4.82 mg/l (96 h) based on geometric mean measured concentrations. The 72 hour E_rC_{10} was 0.97 mg/l based on measured concentrations. The 96 hour E_rC_{10} was 1.00 mg/l based on measured concentrations.

I. MATERIAL AND METHODS

Test item:	Fluxapyroxad (Reg. No. 5 094 351), batch no. COD-000986, purity: 99.5% (tolerance ± 1.0%).
Test species:	Freshwater diatom, *Navicula pelliculosa*, SAG 1050-3; stock obtained from "Sammlung von Algenkulturen", University of Göttingen, Germany.
Test design:	Static system (96 hours); 5 test item concentrations with 5 replicates plus a control with 10 replicates; daily assessment of growth.
Endpoints:	EC_{05}, EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 and 96 hours.
Test concentrations:	Geometric mean measured concentrations: 0 (control), 0.215 mg/l (6.25% dilution), 0.435 mg/l (12.5% dilution), 0.867 mg/l (25% dilution), 1.723 mg/l (50% dilution) and 3.418 mg/l (100%, pure stock solution). The highest test concentration at test initiation reflects the maximum solubility of the test item under test conditions.
Test conditions:	100 mL Erlenmeyer flasks; nutrient solution according to OPPTS 850.5400, respectively, OECD 201 (test volume: 60 mL); pH 8.1 at test initiation, pH 7.60 - 7.99 at test termination; temperature: 22±1 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.
Statistics:	Descriptive statistics; probit analysis for growth rate and yield data after 72 and 96 hours.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for set-ups with concentration levels of 6.25% up to 100% of a saturated stock solution of fluxapyroxad ranged from 0.214 mg/l to 3.409 mg/l at test initiation and from 0.216 mg/l to 3.427 mg/l at test termination. The biological results are based on geometric mean measured concentrations of the test item.

Biological results: No morphological effects on algae were observed in the control group and at any of the test item concentrations tested. The effects on algal growth are summarised in Table 56.

Table 56: Effect of fluxapyroxad on yield and growth rate of fresh water diatom *Navicula pelliculosa* (72 h and 96 h)

% saturated solution	**Control**	**6.25**	**12.5**	**25**	**50**	**100**
Concentration [mg/l] geom. mean measured	**Control**	**0.215**	**0.435**	**0.867**	**1.723**	**3.418**
Inhibition in 72 h (growth rate) [%] [1)]	--	-0.9	0.2	10.1	20.3	27.3
Inhibition in 96 h (growth rate) [%] [1)]	--	0.0	0.6	9.0	20.5	38.7
	Endpoints [mg fluxapyroxad/L] geom. mean measured					
E_rC_{50} (72 h)	8.78 mg/l (extrapolated value)					
E_rC_{10} (72 h)	0.97 (95% confidence limits: 0.80 - 1.13)					
E_rC_{50} (96 h)	4.82 mg/l (extrapolated value)					
E_rC_{10} (96 h)	1.00 (95% confidence limits: 0.94 - 1.05)					

[1)] Inhibition compared to control; negative values indicate stimulated growth.

III. CONCLUSION

In a toxicity test with *Navicula pelliculosa* the E_rC_{50} of fluxapyroxad was determined to be 8.78 mg/l (72h) and 4.82 mg/l (96 h) based on geometric mean measured concentrations. The 72 hour E_rC_{10} was 0.97 mg/l based on measured concentrations. The 96 hour E_rC_{10} was 1.00 mg/l based on measured concentrations.

STUDY 4

Author(s)	Hoffmann F.
Year:	2009(d)
Title:	Effect of BAS 700 F (Reg.No. 5094351) on the growth of the marine diatom *Skeletonema costatum*
Test Guidelines:	OECD 201, EPA 850.5400
Deviations from Guideline:	No
GLP Compliance:	Yes

Executive Summary

The effect of Fluxapyroxad on the growth of the marine diatom *Skeletonema costatum* was investigated in a 96-hour static laboratory study. The following geometric mean measured concentrations were applied: 0 (control), 0.195 mg a.s./l (6.25% dilution), 0.393 mg a.s./l (12.5% dilution), 0.793 mg a.s./l (25% dilution), 1.606 mg a.s./l (50% dilution) and 3.130 mg a.s./l (100%, pure stock solution). Assessment of growth was conducted 24, 48, 72 and 96 h after test initiation.

The biological results are based on geometric mean measured concentrations of the test item. No morphological effects on algae were observed in the control groups and at concentrations up to 1.606 mg a.s./l (geometric mean measured). At the highest concentration of 3.130 mg a.s./l the cells appeared smaller than those in the control.

In a static toxicity test with *Skeletonema costatum* the E_rC_{50} of Fluxapyroxad was determined to be 5.88 mg a.s./l (72 h) and 5.25 mg a.s.//L (96 h) based on geometric mean measured

concentrations. The 72 hour E_rC_{10} was 0.69 mg/l based on measured concentrations. The 96 hour E_rC_{10} 0.64 mg/l based on measured concentrations.

I. MATERIAL AND METHODS

Test item:	Fluxapyroxad (BAS 700 F; Reg. No 5 094 351), batch no. COD-000986, purity: 99.5%.
Test species:	Marine diatom, *Skeletonema costatum*, stock originally obtained from the "UTEX - Culture Collection of Algae", University of Texas, Austin, USA.
Test design:	Static system; test duration 96 hours; 5 test item concentrations plus a dilution water control with 5 replicates per concentration and 10 replicates for the control; daily assessment of growth.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 and 96 hours.
Test concentrations:	Control (dilution water), geometric mean measured concentrations of 0.195 mg a.s./l (6.25% dilution), 0.393 mg a.s./l (12.5% dilution), 0.793 mg a.s./l (25% dilution), 1.606 mg a.s./l (50% dilution) and 3.130 mg a.s./l (100%, pure stock solution).
Test conditions:	100 mL Erlenmeyer flasks; test volume 60 mL; saltwater algal medium (according to OPPTS 850.5400); pH 8.0 at test initiation and pH 8.16 - 8.46 at test termination; temperature: 20 °C±1 °C; initial cell densities 1x 10^4 cells/mL; photoperiod: 14 hours light :10 hours dark; light intensity: about 4300 lux; continuous shaking
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with MS detection.
Statistics:	Descriptive statistics; probit analysis for calculation of EC_x values.

II. RESULTS AND DISCUSSION

Analytical measurements: Mean measured values for set-ups with concentration levels of 6.25% up to 100% of a saturated stock solution of Fluxapyroxad ranged from 0.2 mg/l to 3.1 mg/l at test initiation and from 0.2 mg/l to 3.1 mg/l at test termination. The biological results are based on geometric mean measured concentrations of the test item.

Biological results: No morphological effects on algae were observed in the control groups and at concentrations up to 1.606 mg a.s./l (geometric mean measured). At the highest concentration of 3.130 mg a.s./l the cells appeared smaller than those in the control. The effects on algal growth are summarised in Table 57.

Table 57: Effect of Fluxapyroxad on the growth of the marine diatom *Skeletonema costatum*

Concentration [mg a.s./l] (geometric mean measured)	**Control**	**0.195**	**0.393**	**0.793**	**1.606**	**3.130**
Inhibition in 72 h (growth rate) [%] [1)]	--	-0.3	4.2	11.4	25.0	33.6
Inhibition in 96 h (growth rate) [%] [1)]	--	-0.1	5.5	12.3	25.6	36.4
	Endpoints [mg Fluxapyroxad/L] (geometric mean measured)					
E_rC_{50} (72 h)	5.88 mg/l (extrapolated value)					
E_rC_{10} (72 h)	0.69 (95% confidence limits: 0.60 - 0.77)					
E_rC_{50} (96 h)	5.25 mg/l (extrapolated value)					
E_rC_{10} (96 h)	0.64 (95% confidence limits: 0.58 - 0.70)					

[1)] Inhibition compared to control; negative values indicate stimulated growth.

III. CONCLUSION

In a static toxicity test with *Skeletonema costatum* the E_rC_{50} of Fluxapyroxad was determined to be 5.88 mg a.s./l (72 h) and 5.25 mg a.s.//L (96 h) based on geometric mean measured concentrations. The 72 hour E_rC_{10} was 0.69 mg/l based on measured concentrations. The 96 hour E_rC_{10} 0.64 mg/l based on measured concentrations.

STUDY 5

Author(s)	Hoffmann F.
Year:	2009(f)
Title:	Effect of BAS 700 F (Reg.No. 5094351) on the Growth of *Lemna gibba*
Test Guidelines:	OECD Guideline 221, EPA OPPTS 850.4400 (Draft 1996), ASTM E 1415-91
Deviations from Guideline:	No
GLP Compliance:	Yes

Executive Summary

In a 7-day static toxicity laboratory study, the effect of fluxapyroxad on the growth of the duckweed *Lemna gibba* was investigated. The following geometric mean measured concentrations were applied: 0 (control), 0.215 mg/l (6.25% dilution), 0.438 mg/l (12.5% dilution), 0.869 mg/l (25% dilution), 1.793 mg/l (50% dilution) and 3.425 mg/l (100%, pure stock solution). Assessment of growth and other effects was conducted after 3, 5 and 7 days after test initiation. The percentage growth inhibition, relative to the control, was calculated for each test concentration based upon growth rates and final yield for the parameters frond number and plant dry weight (biomass).

The biological results are based on geometric mean measured concentrations. The duckweed population in the control vessels showed exponential growth, increasing from 11 fronds per vessel to an average of 93 fronds per vessel, corresponding to a 8.5 x multiplication. The dry weight increased from 1.4 mg at test initiation to an average of 14.3 mg per vessel in the control at test termination. No morphological effects on *Lemna gibba* were observed in the control groups and at concentrations up to 0.869 mg/l (geometric mean measured). At the two highest concentration of 1.793 mg a.s./l and 3.425 mg a.s./l the fronds appeared smaller than those in the control.

In a 7-day toxicity test with *Lemna gibba* the E_rC_{50} of fluxapyroxad was determined to be > 3.425 mg/l based on frond number and dry weight (geometric mean measured). The E_rC_{10} was 1.22 mg/l based on frond number and 0.69 mg/l based on dry weight (geometric mean measured).

I. MATERIAL AND METHODS

Test item:	Fluxapyroxad (Reg. No. 5 094 351), batch no. COD-000986, purity: 99.5% (tolerance ± 1.0%).
Test species:	Duckweed (*Lemna gibba* G3), inocula 7 - 10 days old cultures; cultures maintained in-house; stock obtained from "ÖkoTox Moser & Pickl GbR", Stuttgart, Germany.
Test design:	Static system (7 days); 5 test item concentrations with 3 replicates plus a control with 6 replicates; 2 plants with 4 fronds and 1 plant with 3 fronds, total number of fronds at test initiation: 11 per replicate; assessment of growth and other effects after 3, 5 and 7 days of exposure.
Endpoints:	EC_{05}, EC_{10} and EC_{50} with respect to growth rate and yield (based on frond number and dry weight) after exposure over 7 days.
Test concentrations:	Geometric mean measured concentrations: 0 (control), 0.215 mg/l (6.25% dilution), 0.438 mg/l (12.5% dilution), 0.869 mg/l (25% dilution), 1.793 mg/l (50% dilution) and 3.425 mg/l (100%, pure stock solution). The highest test concentration at test initiation reflects the maximum solubility of the test item under test conditions.
Test conditions:	400 ml glass beakers; nutrient solution (20x-APP medium) according to OECD 221 (test volume: 160 mL); pH 7.50 - 7.53 at test initiation, pH 7.72 - 8.75 at test termination; mean temperature: 24.3 °C; continuous light at about 8400 lux.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.
Statistics:	Descriptive statistics; probit analysis for determination of EC_x values for growth rate and yield.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for set-ups with concentration levels of 6.25% up to 100% of a saturated stock solution of fluxapyroxad ranged from 0.233 mg/l to 3.746 mg/l at test initiation and from 0.198 mg/l to 3.131 mg/l at test termination. The biological results are based on geometric mean measured concentrations of the test item.

Biological results: The duckweed population in the control vessels showed exponential growth, increasing from 11 fronds per vessel to an average of 93 fronds per vessel, corresponding to a 8.5 x multiplication. The dry weight increased from 1.4 mg at test initiation to an average of 14.3 mg per vessel in the control at test termination. No morphological effects on *Lemna gibba* were observed in the control groups and at concentrations up to 0.869 mg fluxapyroxad/L (geometric mean measured). At the two highest concentration of 1.793 mg a.s./L and 3.425 mg a.s./L the fronds appeared smaller than those in the control. The effects on growth of *Lemna gibba* are summarised in Table58.

Table 58: Effect of fluxapyroxad on growth rate and development of biomass of duckweed *Lemna gibba* (7 d)

% saturated solution	**6.25**	**12.5**	**25**	**50**	**100**
Concentration [mg/l] (geometric mean measured)	**0.215**	**0.438**	**0.869**	**1.793**	**3.425**
Inhibition after 7 d [%] [1] (growth rate based on frond no.)	-0.7	-1.0	7.1	17.3	41.1
Inhibition after 7 d [%] [1] (growth rate based on dry weight)	0.9	1.3	18.5	21.5	32.3
Inhibition after 7 d [%] [1] (yield based on frond no.)	0.0	0.0	15.9	35.0	66.3
Inhibition after 7 d [%] [1] (yield based on dry weight)	2.7	3.7	38.8	43.7	58.7
Endpoints [mg fluxapyroxad/L] (geometric mean measured)					
E_rC_{50} (7 d) based on frond no.	> 3.425				
E_rC_{10} (7 d) based on frond no.	1.22 (95% confidence limits: 1.09 - 1.35)				
E_rC_{05} (7 d) based on frond no.	0.86 (95% confidence limits: 0.73 -0.98)				
E_yC_{50} (7 d) based on frond no.	2.41 (95% confidence limits: 2.25 – 2.59)				
E_yC_{10} (7 d) based on frond no.	0.77 (95% confidence limits: 0.65 - 0.88)				
E_yC_{05} (7 d) based on frond no.	0.55 (95% confidence limits: 0.45 - 0.66)				
E_rC_{50} (7 d) based on dry weight	> 3.425				
E_rC_{10} (7 d) based on dry weight	0.69 (95% confidence limits: 0.40 - 0.94)				
E_rC_{05} (7 d) based on dry weight	0.35 (95% confidence limits: 0.15 - 0.55)				
E_yC_{50} (7 d) based on dry weight	2.19 (95% confidence limits: 1.71 - 3.06)				
E_yC_{10} (7 d) based on dry weight	0.34 (95% confidence limits: 0.15 - 0.52)				
E_yC_{05} (7 d) based on dry weight	0.20 (95% confidence limits: 0.07 - 0.34)				

[1] Inhibition compared to control; negative values indicate stimulated growth.

III. CONCLUSION

In a 7-day toxicity test with *Lemna gibba* the E_rC_{50} of fluxapyroxad was determined to be > 3.425 mg/l based on frond number and dry weight (geometric mean measured). The E_rC_{10} (frond number) was 1.22 mg/l and the E_rC_{10} (dry weight) 0.69 mg/l (mean measured).

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

No relevant data available.

11.6 Long-term aquatic hazard

With the exception of one study (Minderhout *et al*, 2010) which was not available at the time, studies reviewed under Directive 91/414/EEC and considered reliable are summarised in the DAR. A summary of available valid information on the chronic aquatic ecotoxicity of Fluxapyroxad is presented in Table

The most sensitive chronic endpoint is highlighted. . Summaries of all studies are provided.

Table 59: Summary of relevant information on chronic aquatic toxicity

Species	Guideline and GLP status	Substance	Exposure System	Results	Reference
Fish					
Pimephales promelas Fathead minnow	OECD 210; EPA 72-4 (a); EPA 850.1400 GLP: yes	Fluxapyroxad as BAS 700 F (purity 99.7%)	33 d (Early Life Stage), flow through	**NOEC = 0.0359 mg a.s./l** (mm)	Anonymous (2009z)
Aquatic invertebrates					
Daphnia magna	OECD 211; EPA 850.1300 GLP: yes	Fluxapyroxad as BAS 700 F (purity 99.7%)	21 d, semi-static	NOEC = 0.500 mg a.s./l (n verified)	Janson, 2009b
Americamysis bahia	EPA 850.1350; ASTM E 1191-97 GLP: yes	Fluxapyroxad as BAS 700 F (purity 99.4%)	29 d, flow-through	NOEC = 0.30 mg a.s./l (n verified)	Minderhout *et al*, 2010 *
Algae and aquatic plants					
Pseudokirchneriella subcapitata	OECD 201 GLP: yes	Fluxapyroxad as BAS 700 F (purity 99.5%)	72 h, static	E_rC_{10} = 0.31 mg a.s./l (n verified)	Hoffmann, 2008a, 2009a, 2010
Anabaena flos-aquae	OECD 201; EPA 850.5400 GLP: yes	Fluxapyroxad as BAS 700 F (purity 99.5%)	72 h, static	E_rC_{10} = 1.20 mg a.s./l (mm)	Hoffmann, 2009x
Navicula pelliculosa	OECD 201; EPA 850.5400 GLP: yes	Fluxapyroxad as BAS 700 F (purity 99.5%)	72 h, static	E_rC_{10} = 0.97 mg a.s./l (mm)	Hoffmann, 2009x
Skeletonema costatum	OECD 201; EPA 850.5400 GLP: yes	Fluxapyroxad as BAS 700 F (purity 99.5%)	72 h, static	E_rC_{10} = 0.69 mg a.s./l (mm)	Hoffmann, 2009x
Lemna gibba	OECD 221; ASTM E 1415-91; EPA 850.4400 GLP: yes	Fluxapyroxad as BAS 700 F (purity 99.5%)	7 d, static	$E_rC_{10(\text{frond number})}$ 1.22 mg a.s./l $E_rC_{10(\text{dry weight})}$ 0.69 mg a.s./l (mm)	Hoffmann, 2009x

n: based on nominal concentrations
mm: based on mean measured concentrations
*This study was not available at the time of the DAR preparation and EFSA conclusion. It is included in this CLH report and considered valid.

11.6.1 Chronic toxicity to fish

(Anonymous 2009z)

Executive Summary

The chronic toxicity of Fluxapyroxad (tested as BAS 700 F) to fathead minnow (*Pimephales promelas*) was evaluated in a 33-day early life-stage test under flow-through conditions. Embryos were exposed to a dilution water control and to test item concentrations of 24, 39, 63, 100 and 160 µg/l fluxapyroxad (nominal). The exposure concentration range based on mean measured concentrations was 23.29, 35.88, 67.62, 108.6 and 169.3 µg/l fluxapyroxad.

Hatchability, post-hatch survival rate, time to hatch and swim-up, and growth parameters of fathead minnow embryos were assessed throughout the study.

No concentration-related effect was observed until hatch. The survival from hatch until the end of swim-up (day 7), from the end of swim-up to the end of exposure (day 7 - 33) as well as over the whole exposure period (day 0 - 33) was statistically significantly decreased compared to the control group at the highest tested concentration of 160 µg a.s./l (nominal).

Mortality in the highest concentration group until day 33 was 93% of the test organisms inserted at start of the test compared to a mortality of 16% in the control group.

The time to hatch and swim-up was similar in all test groups including the control. Hatch was observed between days 2 and 5, swim-up between days 4 and 7. No signs of test item-related toxicity or abnormalities were observed in the control and in any of the test item treatment groups.

On day 33 the body weights of the fish in all treatment groups were not statistically significantly different from the control group. However, a trend towards a reduced body weight was observed at the highest tested concentration of 160 µg a.s./l, (nominal), which was most likely not statistically significant because of the low number of survivors in this group.

The total body lengths of the surviving fish at the end of the exposure period were statistically significantly decreased in comparison to the control at test item concentrations of 24, 63 and 100 µg/l (nominal) in the study report. There was no significant decrease in length at 39 µg/l (nominal). In the study report it was considered that the significant effects observed at nominal 24 µg/l (nominal) were not treatment related (see details below). On this basis the NOEC was considered to be 39 µg/l (nominal). The statistically significant decrease of the mean body length in each treatment group was examined under Directive 91/414/EEC. The NOEC was agreed to be 39 µg a.s./l based on nominal concentrations and 35.9 µg a.s./l based on mean measured concentrations

In an early life stage study with fathead minnow (*Pimephales promelas*) the overall NOEC (33 d) for fluxapyroxad was determined to be 39 µg a.s./l based on nominal concentrations and 35.9 µg a.s./l based on mean measured concentrations.

I. MATERIAL AND METHODS

Test item:	Fluxapyroxad (Reg. No. 5 094 351), batch no. COD-000899, purity: 99.7% (tolerance ± 1%).
Test species:	Fathead minnow (*Pimephales promelas*), eggs less than 5 hours old.

Test design:	Flow through system (33 d); 5 test item concentrations plus a dilution water control, 4 replicates per treatment with 25 fertilized eggs in each. Eggs and larvae were exposed in cylindrical glass vessels and were transferred into stainless steel aquaria on day 14. The test solution flowed continuously from the mixing tank into an "udder" which split the test water into 4 equal parts for the 4 replicate test aquaria. On day 33 fish were sacrificed and the body length and weight of surviving larvae were determined. Daily assessment of hatch, swim-up, survival, signs of toxicity and abnormal behaviour.
Endpoints:	NOEC values based on hatch rate, post-hatch survival, toxic signs, growth rates and time spans to hatch and swim-up.
Test concentrations:	Control (dilution water), 24, 39, 63, 100 and 160 µg/l fluxapyroxad (nominal).
Test conditions:	Test vessels: Cylindrical glass vessels: water volume: 1.7 litre; stainless steel aquaria (29 x 21 x 22 cm), water volume: 9 litres. Dilution water: non-chlorinated, filtered drinking water (diluted with deionized water); temperature 25°C-26°C; pH 7.3-8.1; oxygen content 5.1 mg/l-8.4 mg/l; total hardness: 100-103 mg $CaCO_3$/l; conductivity: 234-254 µS; acid capacity: 2.20 mmol/L. Light intensity: 186 lux-430 lux; photoperiod: 16 hours light : 8 hours dark; flow rates: 7.5 l/hour/treatment group, 1.9 l/hour/test vessel. Feeding: freshly hatched *Artemia nauplii* starting at day 6 and commercial fish diet (Tetramin) from day 7 on. Slight aeration from day 18 on.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.
Statistics:	Descriptive statistics; two-sided Dunnett`s test for weight and length data, one-sided Fisher`s exact test for survival data, one-sided Wilcoxon-test for variability between replicates.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in all test item concentrations at weekly intervals until day 33. Mean measured concentrations of fluxapyroxad ranged from 92% to 109% of nominal over the exposure period. All analytically determined concentrations of the test item were within a range of ±20% of the nominal values except for one slight deviation in the 39 µg/l group and two deviations >20% in the highest concentration group. The deviations were considered to have no marked influence on the results obtained until day 33. The following biological results are based on nominal and additionally on mean measured concentrations.

Biological results: No concentration-related effect was observed until hatch. The survival from hatch until the end of swim-up (day 7; Fisher`s exact test and Wilcoxon-test, both $p \leq 0.05$), from the end of swim-up to the end of exposure (day 7 - 33; Fisher`s exact test, $p \leq 0.01$ and Wilcoxon-test, $p \leq 0.05$), as well as over the whole exposure period (day 0 - 33; Fisher`s exact test, $p \leq 0.01$ and Wilcoxon-test, $p \leq 0.05$) was statistically significantly decreased compared to the control group at the highest tested concentration of 160 µg a.s./fluxapyroxad (nominal). Mortality in the highest concentration group until day 33 was 93% of the test organisms inserted at start of the test compared to a mortality of 16% in the control group.

The time to hatch and swim-up was similar in all test groups including the control. Hatch was observed between days 2 and 5, swim-up between days 4 and 7.

No signs of test item-related toxicity or abnormalities were observed in the control and in any of the test item treatment groups.

On day 33 the body weights of the fish in all treatment groups were not statistically significantly different from the control group. However, a trend towards a reduced body weight was observed at the highest tested concentration of 160 µg a.s./l (nominal), which was most likely not statistically significant because of the low number of survivors in this group.

The total body lengths of the surviving fish at the end of the exposure period were statistically significantly decreased in comparison to the control at test item concentrations of 24, 63 and 100 µg/l (nominal) in the study report. There was no significant decrease in length at 39 µg/l (nominal).

In the study report it was considered that the significant effects observed at nominal 24 µg/l (nominal) were not treatment related because:
i) the decrease was close to normal variability for the parameter treatment (control 4 replicate means were 2.5-2.6 cm compared to 24 µg/l 4 replicated means of 2.4-2.5cm);
ii) the high number of survivors in the group (88 compared to 84 in the control) could have slightly influenced growth; and
ii) there was no significant decrease in growth at 39 µg/l (nominal) with 4 replicate means ranging from 2.4 to 2.6 cm.

On this basis the study NOEC was considered to be 39 µg/l (nominal). The study also observed that untypically only body length was affected and not body weight. In addition for the length parameter, less than 10% reduction in length was observed for all treatments when compared to control data.

The statistically significant decrease of the mean body length in each treatment group was examined under Directive 91/414/EEC. The NOEC was agreed to be 39 µg a.s./l based on nominal concentrations and 35.9 µg a.s./l based on mean measured concentrations

The results are summarised in Table 60.

Table 60: Chronic toxicity of fluxapyroxad to fathead minnow (*Pimephales promelas*) in an fish early life stage test (33 d)

Concentration (nominal) [µg/l]	**Control**	**24**	**39**	**63**	**100**	**160**
Concentration (mean measured) [µg/l]	**Control**	**23.29**	**35.88**	**67.62**	**108.6**	**169.3**
Embryo survival until hatch [%]	95	93	91	95	94	95
Survival of larvae from hatch until end of swim-up (day 7) [%]	98	100	99	98	95	**89 ***
Survival of young fish (day 7 - 33) [%]	90	95	96	91	89	**8 ****
Survival from day 0 to test termination (33 d) [%]	84		8688	85	79	**7 ****
Start of hatch [day] End of hatch [day]	2 5	2 5	2 5	2 5	2 5	2 5
Start of swim-up End of swim-up	4 6 - 7	4 6	4 6	4 6	4 6	4 6 - 7
Symptoms	none	none	none	none	none	none
Mean weight (33 d) [mg] Standard deviation in parenthesis	173	164 (36)	190 (56)	173 (53)	173 62)	158 (37)
% of control [1)]	100	95	110	100	100	91
Mean length (33 d) [cm] Standard deviation in parenthesis	2.5	2.4 *** (0.19)	2.5 (0.29)	**2.4 ***** (0.27)	**2.3 ***** (0.31)	2.3 +
% of control [1)]	100	95	99	94	92	91
	Endpoints [µg/l fluxapyroxad]					
$NOEC_{overall}$ (33 d) nominal	39					
$NOEC_{overall}$ (33 d) mean measured	35.9					

Deviations which are considered to be substance-related are printed **bold**.

1) Calculated on the basis of the individual values.

* Statistically significant differences compared to the control (one-sided Fisher`s exact test and one-sided Wilcoxon-test, both $p \leq 0.05$).

** Statistically significant differences compared to the control (one-sided Fisher`s exact test ($p \leq 0.01$) and one-sided Wilcoxon-test, $p \leq 0.05$).

******* Statistically significant differences compared to the control (two-sided Dunnett`s test, $p \leq 0.01$). Refer to text above.

\+ Body length reduced but due to the low numner of survivors (7 fish) it was not statistically significant.

III. CONCLUSION

In an early life stage study with fathead minnow (*Pimephales promelas*) the overall NOEC (33 d) based on effects on mean body length for fluxapyroxad was determined to be 39 µg a.s./l based on nominal concentrations and 35.9 µg a.s./l based on mean measured concentrations.

11.6.2 Chronic toxicity to aquatic invertebrates

STUDY 1

Author(s)	Janson G.-M.
Year:	2009(b)
Title:	Chronic toxicity of BAS 700 F (Reg.No. 5094351) to *Daphnia magna* STRAUS in a 21 day semi-static test
Test Guidelines:	According to OECD 211; EPA 850.1300
Deviations from Guideline:	No
GLP Compliance:	Yes

Executive Summary

In a 21-day semi-static toxicity test, effects of Fluxapyroxad (tested as BAS 700 F) to water fleas (*Daphnia magna*) were examined. Neonates less than 24 hours old were exposed to nominal concentrations of 0.125, 0.25, 0.5, 1.0 and 2.0 mg a.s./l, a control and a solvent control. All treatment groups and the controls consisted of 10 replicates with one parent daphnid in each. After 21 days parent mortality, body length and dry weight and reproductive performance was assessed.

The biological results were based on nominal concentrations. After 21 days of exposure no parent mortality was observed in any of the test item concentrations and the controls. The day of first brood ranged from 8 to 12 and the number of offspring varied between 91and 178. At test end the body length of the adult daphnids ranged from 4.1 to 4.6 mm and the dry weight of the adult daphnids ranged from 0.752 to 1.040 mg. Control and solvent control were pooled for statistical analysis because of non significant differences. Statistically significant effects on reproduction and parent body length compared to the controls were observed at the two highest tested concentrations of 1.0 and 2.0 mg a.s./l. Body dry weight of the parent daphnids was significantly affected at the highest test concentration of 2.0 mg a.s./l.

In a 21-day semi-static toxicity study with *Daphnia magna* the NOEC of fluxapyroxad was determined to be 0.5 mg a.s./l based on verified nominal concentrations.

I. MATERIAL AND METHODS

Test item:	Fluxapyroxad (Reg. No. 5 094 351), batch no. COD-000899, purity: 99.7% (tolerance ± 1%).
Test species:	Water flea (*Daphnia magna* STRAUS), neonates from in-house culture (originally obtained from Institut National de Recherche Chimique Appliquée, France); > 2 < 24 hours old at test initiation.
Test design:	Semi-static system (21 days), 5 test concentrations plus control and solvent control, ten replicates per treatment with one parent daphnid per in each; assessment of parent mortality, body length, dry weight and reproduction after 21 days.
Endpoints:	NOEC, MATC, parent mortality, reproduction, parent length and dry weight.
Test concentrations:	Control, solvent control (acetone), 0.125, 0.25, 0.5, 1.0 and 2.0 mg/l fluxapyroxad (nominal).
Test conditions:	Glass vessels, test volume 50 ml, dilution water “M4” (Elendt medium); temperature: 19.3 °C - 20.9 °C; pH 7.87 - 8.17; oxygen content: 8.4 mg/l - 9.3 mg/l; total hardness: 2.4 - 2.5 mmol/l, conductivity: 607 µS/cm –

	621 µS/cm; light intensity: 212 lux - 718 lux; photoperiod 16 hours light: 8 hours dark; regular feeding with algae (*Desmodesmus subspicatus*), no aeration.
Analytics:	The test item concentrations were analysed using a HPLC-method with mass selective detector.
Statistics:	Descriptive statistics; Analysis of Variance (ANOVA) followed by Dunnett`s test ($p > 0.05$) for reproduction, body length and dry weight; t-test ($p > 0.05$) for comparison of control and solvent control data.

II. RESULTS AND DISCUSSION

Analytical results: Analytical verification of the test item concentrations was conducted in all treatments at day 0, 2, 7, 9, 12, 16 and 21. Mean recoveries of fluxapyroxad were in the range of 81.9% - 103.7% of nominal concentrations during the course of the study. The following biological results are based on nominal test item concentrations.

Biological results: After 21 days of exposure no parent mortality was observed in any of the test item concentrations and the controls. The day of first brood ranged from 8 to 12 and the number of offspring varied between 91and 178. At test end the body length of the adult daphnids ranged from 4.1 to 4.6 mm and the dry weight of the adult daphnids ranged from 0.752 to 1.040 mg. Control and solvent control were pooled for statistical analysis because of non significant differences (t-test; $p > 0.05$). Statistically significant effects on reproduction and parent body length compared to the controls were observed at the two highest tested concentrations of 1.0 and 2.0 mg a.s./l (Dunnett`s test, $p < 0.05$). Body dry weight of the parent daphnids was significantly affected at the highest test concentration of 2.0 mg a.s./l (Dunnett`s test, $\alpha = 0.05$). The results are summarised in Table 61

Table 61: Effects of fluxapyroxad (21 d) on *Daphnia magna* reproduction, growth and parent mortality

Concentration [mg/l] nominal	**Control**	**Solvent control**	**0.125**	**0.25**	**0.5**	**1.0**	**2.0**
Parent mortality [%]	0	0	0	0	0	0	0
Av. offspring/parent	178	166	167	157	170	114 *	91 *
Day of first brood	8-9	8-10	8-9	8-10	8-9	8-12	10-12
Av. body weight [mg]	0.950	1.040	0.916	0.925	0.881	1.040	0.752 *
Av. body length [mm]	4.6	4.6	4.6	4.6	4.6	4.3 *	4.1 *
	Endpoints [mg/l fluxapyroxad] (nominal)						
$NOEC_{overall}$ (21 d)	0.5						

* Statistically significant effects compared to the pooled control (Dunnett`s test; $p < 0.05$).

III. CONCLUSION

In a 21-day semi-static toxicity study with *Daphnia magna* the NOEC of fluxapyroxad was determined to be 0.5 mg a.s./l based on verified nominal concentrations and effects on average body length and reproduction.

STUDY 2

Author(s)	Minderhout T. *et al*.
Year:	2010
Title:	BAS 700 F: A flow-through life-cycle toxicity test with the saltwater mysid (*Americamysis bahia*)
Test Guideline(s):	EPA 850.1350
Deviations from Guideline:	No
GLP Compliance:	Yes

This study was not available at the time of the DAR preparation and EFSA conclusion. It is included in this CLH report and considered valid.

Executive Summary

The chronic toxicity of Fluxapyroxad (tested as BAS 700 F) to saltwater mysids (*Americamysis bahia*) was evaluated in a 29-day life cycle test under flow-through conditions. Mysids were exposed to a dilution water control, a solvent control and to nominal test item concentrations of 0.075, 0.15, 0.30, 0.60 and 1.2 mg a.s./l (corresponding to mean measured concentrations of 0.069, 0.14, 0.28, 0.54, 0.99 mg a.s./l). Survival, reproduction and symptoms of toxicity were assessed throughout the study. Length and dry weight of first-generation mysids were determined at test termination. The biological results are based on verified nominal concentrations.

After 15 days of exposure, mean survival rates of juvenile mysids in the test item treatment groups ranged from 95% to 100%. At test termination, mean survival rates of adult mysids in the test item treatment groups ranged from 91% to 98%. The survival of juvenile and adult mysids was not statistically significantly reduced compared to the pooled control in any test item treatment group.

The day of first brood ranged from 16 to 29 and the mean number of offspring per reproductive day in the test item treatment groups varied between 0.108 and 0.504.

Statistically significant effects on reproduction compared to the pooled control were observed at the highest tested concentrations of 0.99 mg a.s./l. A decrease in reproduction in the next lowest treatment of 0.54 mg a.s./l treatment group did not show a statistically significant difference compared to the pooled control. However, as statistically significant effects were observed for weight and length for the 0.54 mg a.s./l treatment, the study authors considered a conservative approach whereby the observed decrease could be considered biologically significant and, consequently, it was used to determine the NOEC for reproduction.

The 29-day study NOEC for reproduction was therefore considered to be 0.3 mg a.s./l based on verified nominal concentrations. Given that statistically based NOECs are available for length and weight also at 0.3 mg a.s./l, this choice NOEC for reproduction is not considered further in this CLH report.

At test end, the mean total length and dry weight of male adult mysids in the test item treatment groups ranged from 7.44 mm to 7.81 mm and from 0.84 mg to 0.98 mg, respectively. Statistically significant effects on mean total length and dry weight of male adult mysids compared to the pooled control were observed at the highest tested concentrations of 0.99 mg a.s./l for length and at 0.54 and 0.99 mg a.s./l for weight.

At test end, the mean total length and dry weight of female adult mysids in the test item treatment groups ranged from 7.36 mm to 7.94 mm and from 0.89 mg to 1.23 mg, respectively. Statistically

significant effects on mean total length and dry weight of female adult mysids compared to the pooled control were observed at the two highest tested concentrations of 0.54 and 0.99 mg a.s./l for both length and weight.

In a 29-day flow-through toxicity study with saltwater mysids (*Americamysis bahia*), the overall NOEC of Fluxapyroxad was determined to be 0.3 mg a.s./l based on verified nominal concentrations for weight (male and female) and length (female). A conservative NOEC for reproduction of 0.3 mg a.s./l is also available.

I. MATERIAL AND METHODS

Test item:	Fluxapyroxad (BAS 700 F; Reg. No 5 094 351), batch no. COD-001026, purity: 99.4%.
Test species:	Test species: Saltwater mysid (*Americamysis bahia*), neonates, age: less than 24 hours; source: in-house cultures.
Test design:	Flow-through system (29 days), 5 test concentrations plus control and solvent control, with four replicates each; 15 neonate mysids at test initiation; on day 15 male and female adults were paired in each treatment and control group, with a maximum of five reproductive pairs per replicate; assessment of mortality and symptoms of toxicity were conducted daily; assessment of body length and dry weight of all surviving first-generation mysids at test termination; reproduction was monitored through termination on day 29.
Endpoints:	NOEC, mortality, reproduction, parent length and dry weight.
Test concentrations:	Control (dilution water), solvent control (0.1 ml dimethylformamide/l), 0.075, 0.15, 0.30, 0.60 and 1.2 mg a.s./l (nominal); corresponding to mean measured concentrations of 0.069, 0.14, 0.28, 0.54, 0.99 mg a.s./l.
Test conditions:	Test chambers: from test initiation until day 15: 9 litre glass aquaria, test volume approx. 2. litres containing four test compartments: 2 litre glass beakers (12 cm in diameter and 19 cm height) with nylon mesh covered holes on opposite sides of the container. From day 15 on: 19 litre glass aquaria, test volume approx. 14.5 litres, containing up to five reproduction compartments: petri dishes of 10 cm diameter with sides of nylon mesh screen; dilution water: filtered, aerated, sterilized and diluted seawater; flow rates: juvenile test chamber: at least 18 volume additions per 24 hours, adult test chamber: at least 6 volume additions per 24 hours; salinity: 19 - 21‰; temperature: 24.1°C - 26.2°C; pH 7.8 - 8.1; oxygen content: 4.5 - 7.4 mg/l; total hardness: 2.4 mmol//l - 2.5 mmol/L; photoperiod 14 h light : 10 h dark; light intensity: 106 lux at test initiation; feeding live brine shrimp nauplii (*Artemia* sp.) up to four times per day.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with variable wavelength detection.
Statistics:	Descriptive statistics; Fisher's Exact test ($p \leq 0.05$) for survival data, Dunnett`s test (one-tailed, $p \leq 0.05$) for reproduction and growth data; t-test ($p > 0.05$) for comparison of control and solvent control data.

II. RESULTS AND DISCUSSION

Analytical results: Analytical verification of test item concentration was conducted in each concentration at test initiation, on days 7, 10, 14, 21, 28 and at test termination. Mean measured concentrations of Fluxapyroxad ranged from 83% - 93% of nominal concentrations throughout the test. As measured concentrations confirmed correct application of the test substance, the following biological results are based on nominal concentrations.

Biological results: Since there were no statistically significant differences in survival, reproduction and growth between the negative and solvent control groups, the control data were pooled for comparisons to the treatment groups.

After 15 days of exposure, mean survival rates of juvenile mysids in the test item treatment groups ranged from 95% to 100%. At test termination, mean survival rates of adult mysids in the test item treatment groups ranged from 91% to 98%. The survival of juvenile and adult mysids was not statistically significantly reduced compared to the pooled control in any test item treatment group (Fisher's Exact test, $p > 0.05$).

The day of first brood ranged from 16 to 29 and the mean number of offspring per reproductive day in the test item treatment groups varied between 0.108 and 0.504.

Statistically significant effects on reproduction compared to the pooled control were observed at the highest tested concentrations of 0.99 mg a.s./l. A decrease in reproduction in the next lowest treatment of 0.54 mg a.s./l treatment group did not show a statistically significant difference compared to the pooled control. However, as statistically significant effects were observed for weight and length for the 0.54 mg a.s./l treatment, the study authors considered a conservative approach whereby the observed decrease could be considered biologically significant and, consequently, it was used to determine the NOEC for reproduction.

The 29-day study NOEC for reproduction was therefore considered to be 0.3 mg a.s./l based on verified nominal concentrations. Given that statistically based NOECs are available for length and weight also at 0.3 mg a.s./l, this choice of NOEC for reproduction is not considered further in this CLH report.

At test end, the mean total length and dry weight of male adult mysids in the test item treatment groups ranged from 7.44 mm to 7.81 mm and from 0.84 mg to 0.98 mg, respectively. Statistically significant effects on mean total length and dry weight of male adult mysids compared to the pooled control were observed at the highest tested concentrations of 0.99 mg a.s./l for length and at 0.54 and 0.99 mg a.s./l for weight (Dunnett`s test, $p \leq 0.05$).

At test end, the mean total length and dry weight of female adult mysids in the test item treatment groups ranged from 7.36 mm to 7.94 mm and from 0.89 mg to 1.23 mg, respectively. Statistically significant effects on mean total length and dry weight of female adult mysids compared to the pooled control were observed at the two highest tested concentrations of 0.54 and 0.99 mg a.s./l for length and weight (Dunnett`s test, $p \leq 0.05$).

The results are summarised in the following table.

Table 62: Chronic toxicity (29 d) of Fluxapyroxad to saltwater mysids (*Americamysis bahia*)

Concentration [mg a.s./l] (nominal)	**Control**	**Solvent control**	**Pooled control**	**0.075**	**0.15**	**0.30**	**0.60**	**1.2**
Concentration [mg a.s./l] (mean measured)	**--**	**--**	**--**	**0.069**	**0.14**	**0.28**	**0.54**	**0.99**
Juvenile survival until pairing (day 15) [%]	95	98	97	98	95	98	100	98
Parent survival until test termination (day 29) [%]	96	96	96	92	98	91	92	94
Av. offspring/ reproductive day	0.388	0.473	0.431	0.504	0.315	0.362	0.197 [1)]	0.108 *
Day of first brood	16 - 25	16 - 25	--	16 - 28	16 - 27	16 - 28	16 - 29	19 - 29
Av. male body weight (day 29) [mg]	1.02	1.00	1.01	0.98	0.97	0.94	0.84 *	0.86 *
Av. female body weight (day 29) [mg]	1.22	1.34	1.28	1.23	1.18	1.12	1.01 *	0.89 *
Av. male body length (day 29) [mm]	7.78	7.72	7.75	7.81	7.79	7.69	7.61	7.44 *
Av. female body length (day 29) [mm]	7.88	7.99	7.93	7.94	7.83	7.84	7.71 *	7.36 *
	Endpoints [mg a.s./l] (nominal)							
$NOEC_{overall}$ (29 d)	0.30							

* Statistically significant effects on growth and reproduction compared to the pooled control (Dunnett`s test; one-tailed, $p \leq 0.05$).

[1)] While the decrease in reproduction in the 0.54 mg a.i./L treatment group was not statistically significant in comparison to the pooled control (Dunnett one-tailed test, $p \leq 0.05$), given the statistically significant effects in length and weight the study authors considered it conservative to consider the decrease potentially biologically significant.

III. CONCLUSION

In a 29-day flow-through toxicity study with saltwater mysids (*Americamysis bahia*), the overall NOEC of Fluxapyroxad was determined to be 0.3 mg a.s./l based on verified nominal concentrations for weight (male and female) and length (female). A conservative NOEC for reproduction of 0.3 mg a.s./l is also available.

11.6.3 Chronic toxicity to algae or other aquatic plants

See study summaries for algae and aquatic plants provided under 11.5.3.

11.6.4 Chronic toxicity to other aquatic organisms

No relevant data available.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Aquatic acute toxicity data are available for fish, invertebrates, algae and aquatic plants. The most acutely sensitive trophic group is fish with a 96-hour LC_{50} value for *Cyprinus carpio* of 0.290 mg/l. It is noted that the lowest E_rC_{50} for algae (0.7 mg/l for *P. subcapitata*) is in the same toxicity range of 0.1 to 1.0 mg/l.

On the basis of this acute fish endpoint being in the range 0.1 mg/l < $L(E)C_{50}$ ≤1.0 mg/l, Fluxapyroxad should be classified as Aquatic Acute 1 (H400) with an acute M-factor of 1.

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

The degree of biodegradation of Fluxapyroxad in the carbon dioxide evolution test was <10% after an exposure period of 28 days. Fluxapyroxad is considered hydrolytically and photolytically stable. In an irradiated water-sediment simulation study, minimal mineralisation was observed with whole system DT_{50} values calculated to be 355 to 444 days at 12°C.

Overall, the degradation information does not provide sufficient data to show Fluxapyroxad is ultimately degraded within 28 days (equivalent to a half-life < 16 days) or transformed to non-classifiable products. Consequently, Fluxapyroxad has to be considered as 'not rapidly degradable'.

Fluxapyroxad has a log K_{OW} value of 3.13 (at pH 7) which is below the CLP criterion of 4. An experimental fish bioconcentration study reported whole fish BCFs less than the CLP criterion of 500 for both Fluxapyroxad and total radioactive residue: (whole fish steady state lipid normalised (to 5%) BCFs were 46 to 47 l/kg based on parent and 110 to 119 l/kg based on TRR. The BCF study also demonstrated a moderate metabolic transformation of Fluxapyroxad to metabolites of higher polarity after uptake in Bluegill sunfish. The low bioconcentration factors and the rapid elimination of radioactive residues demonstrate a low bioconcentration potential of Fluxapyroxad.

Aquatic chronic/long-term toxicity data are also available for fish, invertebrates, algae and aquatic plants, with fish being the most sensitive species (33 d FELS NOEC = 0.0359 mg/l for *Pimephales promelas*).

Considering this data and that Fluxapyroxad is not rapidly degradable, the substance should be classified as Aquatic Chronic 1 (H410). Given the lowest chronic NOEC is in the range 0.01 < NOEC ≤ 0.1 mg/l a chronic M-factor of 1 is required. It is 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.* Should new data become available, the chronic M-factor may need to be reconsidered.

It is noted that while not considered substance related, statistically significant effects for length were observed in the chronic fish FELS study at the lowest treatment. For comparison, the surrogate approach has been applied to the lowest acute toxicity to fish endpoint (also the lowest acute endpoint overall). This also results in an M-factor of 1.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Aquatic Acute 1: H400 'Very toxic to aquatic life'
Acute M-factor: 1

Aquatic Chronic 1: H410 'Very toxic to aquatic life with long lasting effects'
Chronic M-factor: 1

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 / guide-line, 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
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} ≤ 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 ≤ 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 ra-

dioactivity 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 bio-accumulation. 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} ≤ 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 ≤ 0.1 mg/L range, the **chronic M-factor is 1.**

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

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

No specific data available.

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.

Fluxapyroxad does not contain any halogen functionality other than fluorine.

12.1.2 Comparison with the CLP criteria

A substance is considered hazardous to the ozone layer 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.

Although no specific data have been provided for this hazard, considering the chemical structure and other available information on the physcio-chemcial properties, fluxapyroxad is not expected to be hazardous to stratospheric ozone.

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

Not classified – Conclusive but not sufficient for classification

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**.

13 ADDITIONAL LABELLING

Additional labelling is not required.

14 REFERENCES

A number of references have been removed for reasons of confidentiality. In the text, these are referred to as "Anonymous (YEARx)". Full details of these references can be found in the confidential annex for to this report.

EFSA conclusion; EFSA Journal 2012;10(1):2522

Draft Assessment Report – DAR – Volume 3, Annex B2: Physical and Chemical Properties – January 2011

Draft Assessment Report – DAR – Volume 3, Annex B6a: Toxicology and Metabolism – January 2011

Draft Assessment Report – DAR – Volume 3, Annex B8: Environmental Fate and Behaviour – January 2011

Draft Assessment Report – DAR – Volume 3, Annex B9: Ecotoxicology

Budinsky R.A., LeCluyse E.L., Ferguson S.S, Rowlands J.C., Simon T.(2010). Human and rat primary hepatocyte CYP1A1 and 1A2 induction with 2,3,7,8-tetrachlorodibenzo-p-dioxin, 2,3,7,8-tetrachlorodibenzofuran, and 2,3,4,7,8-pentachlorodibenzofuran. Toxicol. Sci, 118(1), 224–35

Dellarco, VL, McGregor, D, Sir Berry, C, Cohen, SM, Boobis, AR (2006). Thiazopyr and thyroid disruption: Case study within the context of the 2006 IPCS human relevance framework for analysis of a cancer mode of action. Crit Rev Toxicol. 36: 793-801

Meek, ME, Bucher, JR, Cohen, SM, Dellarco, VL., Hill, RN, Lehmann-Mckeeman, LD, Longfellow, DG, Pastoor, T, Seed, J. Patton, DE. (2003). A framework for human relevance analysis of information on carcinogenic modes of ac-tion. Crit Rev Toxicol. 33: 591-654

Curran, PG, DeGroot, LJ (1991). The effect of hepatic enzyme-inducing drugs on thyroid hormones and the thyroid gland. Endocr Rev. 12: 135-150

Dohler, KD, Wong, CC, von zur Muhlen, A. (1979). The rat as model for the study of drug effects on thyroid function: Consideration of methodological problems. Pharmacol Therap, 5: 305-318

Hill, RN, Erdreich, LS, Paynter, OE, Roberts, PA, Rosenthal, SL, Wilkinson, CF. (1989). Thyroid follicular-cell car-cinogenesis. Fund Appl Pharmacol. 12: 629-697

Specific Reference

Author(s)	**Date** **Year /** **Month** **/ Day**	**Title** **Source** **BASF DocID** **GLP or GEP status** **Published or not**	**Owner**

Author(s)	**Date** **Year / Month / Day**	**Title** **Source** **BASF DocID** **GLP or GEP status** **Published or not**	**Owner**
Kroehl T.	2006a	Physical properties of Reg.No. 5 094 351 - Pure active ingr dient BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 2006/1036276 Yes unpublished	BASF
Kroehl T.	2008a	Physical and chemical properties of BAS 700 F (Reg.No. 5 094 351) TC - Accelerated storage stability up to 2 weeks at 54°C BASF SE; Limburgerhof; Germany Fed.Rep. 2008/1014896 Yes unpublished	BASF
Wilfinger W.	2008a	Water solubility of BAS 700 F (Reg.No. 5 094 351) at 20°C Eurofins-GAB GmbH; Niefern-Oeschelbronn; Germany Fed.Rep. 2007/1056999 Yes unpublished	BASF
Wilfinger W.	2008b	Partition coefficient of BAS 700 F (Reg.No. 5 094 351) (HPLC method) Eurofins-GAB GmbH; Niefern-Oeschelbronn; Germany Fed.Rep. 2007/1057001 Yes unpublished	BASF
Loehr S.	2008a	Evaluation of physical and chemical properties according to Directive 94/37/EC (67/548/EC Annex V) BASF SE; Ludwigshafen/Rhein; Germany Fed.Rep. 2008/1070100 Yes unpublished	BASF
Wilfinger W.	2008c	Dissociation constant of BAS 700 F (Reg.No. 5 094 351) in water Eurofins-GAB GmbH; Niefern-Oeschelbronn; Germany Fed.Rep. 2007/1057000 Yes unpublished	BASF
Ferreira L.A.	2009a	BAS 700 F - Determinacao da corrosividade em metais TECAM - Tecnologia Ambiental Ltda.; Sao Paulo; Brazil 2009/3000159 Yes unpublished	BASF

Author(s)	**Date** **Year / Month / Day**	**Title** **Source** **BASF DocID** **GLP or GEP status** **Published or not**	**Owner**
Wilfinger W.	2008d	Solubility of BAS 700 F (Reg.No. 5 094 351) in organic solvents Eurofins-GAB GmbH; Niefern-Oeschelbronn; Germany Fed.Rep. 2007/1057003 Yes unpublished	BASF
Schulz M., Landsiedel R.	2008a	BAS 700 F - Salmonella typhimurium/Escherichia coli reverse mutation assay (standard plate test and preincubation test) BASF SE; Limburgerhof; Germany Fed.Rep. 2008/1028479 Yes unpublished	BASF
Schulz M., Landsiedel R.	2008b	BAS 700 F - In vitro chromosome aberration assay in V79 cells BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 2007/1023153 Yes unpublished	BASF
Schulz M., Landsiedel R.	2007a	In vitro gene mutation test in CHO cells (HPRT locus assay) with Reg.No. 5094351 BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 2007/1020715 Yes unpublished	BASF
Elcombe B. et al	2014	Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: A case study with Phenobarbital as a model constitutive androstane receptor (CAR) activator. Critical Reviews in Toxicology, 44, 64-82	Published paper
Elcombe B.	2016a	BAS 700 F - Enzyme and DNA-Synethesis induction in cultured Sprague Dawley male wild-type (WT) and car knock-out (CARGO) rat hepatocytes CXR Biosciences Ltd.; Dundee DD1 5JJ; United Kingdom 2016/1091503 No unpublished	BASF
Elcombe B.	2016c	BAS 700 F (Fluxapyroxad) - Enzyme and DNA-synthesis induction in cultured male Sprague Dawley wild-type (WT) and CAR Knock-out (CAR KO) rat hepatocytes CXR Biosciences Ltd.; Dundee DD1 5JJ; United Kingdom 2015/1243689 No unpublished	BASF

Author(s)	**Date** **Year / Month / Day**	**Title** **Source** **BASF DocID** **GLP or GEP status** **Published or not**	**Owner**
Elcombe B.	2016d	BAS 700 F (Fluxapyroxad) - Enzyme and DNA-synthesis induction in cultured male Wistar HAN rat hepatocytes CXR Biosciences Ltd.; Dundee DD1 5JJ; United Kingdom 2016/1084305 No unpublished	BASF
Elcombe B.	2016e	BAS 700 F (Fluxapyroxad) - Enzyme and DNA-synthesis induction in cultured male human hepatocytes - Assessment of two separate donors CXR Biosciences Ltd.; Dundee DD1 5JJ; United Kingdom 2015/1184808 No unpublished	BASF
Kamp H., Kaufmann W.	2009a	Amendment No. 1: BAS 700 F - Repeated dose toxicity study in Wistar rats; Administration in the diet for 4 weeks BASF SE; Ludwigshafen/Rhein; Germany Fed.Rep. 2009/1110800 Yes unpublished	BASF
Schwarz H.	2008	BAS 700 F: Determination of the biodegradability in the CO2-evolution test BASF SE; Ludwigshafen/Rhein; Germany Fed.Rep. 2008/1028082 Yes unpublished	BASF
Hassink J.	2009a	BAS 700 F: Aqueous hydrolysis at four different pH values BASF SE; Limburgerhof; Germany Fed.Rep. 2009/1049061 Yes unpublished	BASF
Ebert D.	2009a	Degradation of BAS 700 F in water/sediment systems under aerobic conditions BASF SE; Limburgerhof; Germany Fed.Rep. 2009/1004082 Yes unpublished	BASF
Hassink J.	2009b	Aqueous photolysis of BAS 700 F BASF SE; Limburgerhof; Germany Fed.Rep. 2009/1031228 Yes unpublished	BASF
Hassink J.	2009c	Photolysis of M700F007 (metabolite of BAS 700 F) in sterile natural water BASF SE; Limburgerhof; Germany Fed.Rep. 2009/1070298 Yes unpublished	BASF

Author(s)	**Date** **Year / Month / Day**	**Title** **Source** **BASF DocID** **GLP or GEP status** **Published or not**	**Owner**
Hassink J., Stephan A.	2009a	Determination of the adsorption/desorption behaviour of BAS 700 F on different soils BASF SE; Limburgerhof; Germany Fed.Rep. 2009/1065633 Yes unpublished	BASF
Janson G.-M.	2009a	Acute toxicity of BAS 700 F (Reg. No. 5094351) to Daphnia magna STRAUS in a 48 hour static test BASF SE; Limburgerhof; Germany Fed.Rep. 2008/1028252 Yes unpublished	BASF
Gallagher S.P. et al.	2009a	BAS 700 F: A 96-hour static acute toxicity test with the saltwater mysid (Americamysis bahia) Wildlife International Ltd.; Easton MD; United States of America 2009/7000069 Yes Unpublished	BASF
Gallagher S.P. et al.	2009b	BAS 700 F: A 96-hour shell deposition test with the eastern oyster (Crassostrea virginica) Wildlife International Ltd.; Easton MD; United States of America 2009/7000165 Yes unpublished	BASF
Hoffmann F.	2008a	Effect of BAS 700 F (Reg.No. 5094351) on the growth of the green alga Pseudokirchneriella subcapitata BASF SE; Limburgerhof; Germany Fed.Rep. 2008/1022788 Yes unpublished	BASF
Hoffmann F.	2009a	Report amendment No. 1: Effect of BAS 700 F (Reg.No. 5094351) on the growth of the green alga Pseudokirchneriella subcapitata BASF SE; Limburgerhof; Germany Fed.Rep. 2009/1015272 Yes unpublished	BASF
Hoffmann F.	2010	Amendment No. 2 - Effect of BAS 700 F (Reg.No. 5094351) on the growth of the green alga Pseudokirchneriella subcapitata BASF SE; Limburgerhof; Germany Fed.Rep. 2010/1016358 Yes unpublished BASF	BASF

Author(s)	**Date** **Year / Month / Day**	**Title** **Source** **BASF DocID** **GLP or GEP status** **Published or not**	**Owner**
Hoffmann F.	2009b	Effect of BAS 700 F (Reg.No. 5094351) on the growth of the blue-green alga Anabaena flos-aquae BASF SE; Limburgerhof; Germany Fed.Rep. 2009/1079883 Yes unpublished	BASF
Hoffmann F.	2009c	Effect of BAS 700 F (Reg.No. 5094351) on the growth of the fresh water diatom Navicula pelliculosa BASF SE; Limburgerhof; Germany Fed.Rep. 2009/1079885 Yes unpublished	BASF
Hoffmann F.	2009d	Effect of BAS 700 F (Reg.No. 5094351) on the growth of the marine diatom Skeletonema costatum BASF SE; Limburgerhof; Germany Fed.Rep. 2009/1079884 Yes unpublished	BASF
Hoffmann F.	2009f	Effect of BAS 700 F (Reg.No. 5094351) on the growth of Lemna gibba BASF SE; Limburgerhof; Germany Fed.Rep. 2009/1086122 Yes unpublished	BASF
Janson G.-M.	2009b	Chronic toxicity of BAS 700 F (Reg.No. 5094351) to Daphnia magna STRAUS in a 21 day semi-static test BASF SE; Limburgerhof; Germany Fed.Rep. 2008/1055084 Yes unpublished	BASF
Minder-hout T. et al.	2010	BAS 700 F: A flow-through life-cycle toxicity test with the saltwater mysid (Americamysis bahia) Wildlife International Ltd.; Easton MD; United States of America 2009/7006424 Yes unpublished	BASF

Additional references

Curran, PG, DeGroot, LJ (1991). The effect of hepatic enzyme-inducing drugs on thyroid hormones and the thyroid gland. Endocr Rev. 12: 135-150.

Dellarco, VL, McGregor, D, Sir Berry, C, Cohen, SM, Boobis, AR (2006). Thiazopyr and thyroid disruption: Case study within the context of the 2006 IPCS human relevance framework for analysis of a cancer mode of action. Crit Rev Toxicol. 36: 793-801.

Dohler, KD, Wong, CC, von zur Muhlen, A. (1979). The rat as model for the study of drug effects on thyroid function: Consideration of methodological problems. Pharmacol Therap, 5: 305-318

Elcombe, C.R., Peffer, R.C., Wolf, D.C., Bailey, J., Bars, R., Bell, D., Cattley, R.C., Ferguson, S.S., Geter, D., Goetz, A., Goodman, J.I., Hester, S., Jacobs, A., Omiecinski, C.J., Schoeny, R., Xie, W., Lake, B.G., 2014. Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: a case study with phenobarbital as a model constitutive androstane receptor (CAR) activator. Crit. Rev. Toxicol. 44, 64–82.

Hill, RN, Erdreich, LS, Paynter, OE, Roberts, PA, Rosenthal, SL, Wilkinson, CF. (1989). Thyroid follicular-cell carcinogenesis. Fund Appl Pharmacol. 12: 629-697.

Lake (2018) Human relevance of rodent liver tumour formation by constitutive androstane receptor (CAR) activators. Toxicol. Res.,7: 697-717

Meek, ME, Bucher, JR, Cohen, SM, Dellarco, VL., Hill, RN, Lehmann-Mckeeman, LD, Longfellow, DG, Pastoor, T, Seed, J. Patton, DE. (2003). A framework for human relevance analysis of information on carcinogenic modes of action. Crit Rev Toxicol. 33: 591-654.

15 ANNEXES

Confidential Reference Annex