Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Adopted

30 November 2018

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 3-(DIFLUOROMETHYL)-1-METHYL-N-(3',4',5'-TRIFLUOROBIPHENYL-2-YL)PYRAZOLE-4-CARBOXAMIDE; FLUXAPYROXAD

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: 3-(difluoromethyl)-1-methyl-N-(3',4',5'-trifluorobiphenyl-2-yl)pyrazole-4-carboxamide; fluxapyroxad
EC number: -
CAS number: 907204-31-3
Dossier submitter: The United Kingdom

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
04.05.2018	France		MemberState	1
Comment received				
-According to the DAR of the substance, CA name of the substance is 1H-Pyrazole-4-carboxamide, 3-(difluoromethyl)-1-methyl-N-(3',4',5'-trifluoro[1,1'-biphenyl]-2-yl)- -The molecular mass is 381.31g/mol not 381.30g/mol -According to the DAR of the substance, the minimum purity of the active substance is 950 g/kg not 980g/kg.				
Dossier Submitter's Response				
Thank you for your comments regarding the identity of fluxapyroxad. The primary identifier used in the CLH report was advised by ECHA during the dossier submission process: **3-(difluoromethyl)-1-methyl-N-(3',4',5'-trifluorobiphenyl-2-yl)pyrazole-4-carboxamide; fluxapyroxad**. The molecular mass should be as quoted in the DAR: **381.31 g/mol** The minimum purity of the substance should be as quoted in the DAR: **950 g/kg**				
RAC's response				
Noted.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
09.05.2018	Germany		MemberState	2

Comment received

On page 35 the following statement has been made "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." What is the basis for this conclusion given that in the following sentence it has been stated that "fluxapyroxad may be slightly cytotoxic but only at the highest dose tested (300 µM)?"

DE-CA is not convinced that a carcinogenic affect in humans can be ruled out. Firstly, on page 51 and subsequently on page 52 the argument is made that the lack of hepatocellular proliferation observed in cultured human hepatocytes "may indicate a lack of human relevance of the liver tumour findings seen in rats." Presumably this is based on the lack of change in the replicative DNA synthesis measured in the new study by Elcombe (2016e) and reported on page 40. Given that the positive control, phenobarbital induced only a 1.2 fold increase, it is questionable as to whether this is a valid study/method.

Secondly, a mode of action via activation of the AhR receptor cannot be ruled out due to evidence of CYP1A activation (increased EROD activity), as seen in the in vitro rat hepatocyte assays (Elcombe, 2016c, 2016d).

Furthermore, despite the assurances made in the review by Elcombe et al (2014), the molecular initiating event that causes activation of the CAR receptor is still unknown and there is significant cross-talk between the molecular pathways, e.g. CAR, PXR and AhR.

Dossier Submitter's Response

Thank you for your comments. We note the conclusions of the DE-CA, but remain of the opinion that the most plausible MoA for rat liver tumour induction is via CAR/PXR activation and that there is sufficient evidence to conclude that they are not relevant for humans.

Addressing the specific points:
P 35 - inhibition of CYP2B activity by Fluxapyroxad; Decreased CYP2B enzyme activity (measured by PROD and BROD) was reported in the in vitro study (Elcombe B. 2016a) utilising hepatocytes from wild type and CAR knock-out Sprague-Dawley rats. The study reported in table 25 on page 33 "in vitro studies with rat microsomes" supports the fact that fluxapyroxad inhibits CYP2B activity. In this study an IC50 of 0.87 µM for CYP 2B inhibition was observed.

P51, 52 and p40; lack of hepatocellular proliferation observed in cultured human hepatocytes; The positive control in the study was EGF. Treatment with EGF indicated that the cells were able to proliferate and supports the validity of the study.

Therefore we consider the human hepatocyte study to provide reliable information on the Human relevance of rat liver tumours induced via a CAR/PXR MoA.

Activation of AhR; We agree that there is an EROD response, suggesting some activation of the AhR. However, the lack of a response in the CAR KO hepatocytes suggests that although there may be some cross reactivity, there is no increase in DNA synthesis,

something that would be expect if AhR activation was in part responsible for tumour induction. Therefore, fluaxapyroxad appears to be a weak activator of the AhR at most.

Overall, we remain convinced the most plausible MoA for rat liver tumour induction is via CAR/PXR activation, and there is sufficient information to conclude on human health.

RAC's response

RAC agrees with the DS.

The enzyme inhibition study is a curious imvestigation and serves to highlight a potential confounder when measuring induction via enzyme activity. It suggests that the ability of the test substance to substantiate a MoA such as activation of a nuclear receptor may be underestimated if the expression signature or downstream effects of that receptor is confined to enzyme activity alone. It illustrates the importance of having several metrics such as mRNA levels and other enzymes or components of metabolism, histopathology and organ effects in evaluating the response of the molecular target.

Activation of AhR: A typical feature of studies showing activation of CAR by a test substance is a low level increase in CYP1A expression or EROD activity suggesting AhR binding (this is seen even with the CAR positive control phenobarbital). AhR activation is not nesessarily an indicator of a carcinogenic potential. Natural ligands of the receptor play an important role in determining cellular homeostatsis and protection from pro-apoptotic oxidative events within the cell. What we do know is that compounds with significant carcinogenic potential have far greater increases in EROD activity and CYP1A mRNA expression levels as a response to AhR agonist binding than is shown in the mechanistic studies with fluxapyroxad. It is highly unlikely that fluxapyroxad is a strong agonist of the AhR. The results showing mRNA induction illustrate that increases in CYP1A mRNA expression are similar to that for PB and are far weaker responses than those seen for CYP2B mRNA expression.

Overall RAC agrees with the DS and considers fluxapyroxad interacts with CAR to promote rat liver tumours in a rodent MoA considered to have little relevance for humans.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
09.05.2018	Germany		MemberState	3

Comment received

In the last paragraph on page 64, the high dose for the rabbit repro-tox study (Anon, 2009k) is stated as being 1000 mg/kg, yet in the preceding table the highest dose was 60 mg/kg. Please clarify/correct.

Dossier Submitter's Response

Thank you for your comment. The 1000 mg/kg/day is an error, it should be 60 mg/kg/day,

RAC's response

Noted.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
04.05.2018	France		MemberState	4
Comment received				
FR agrees with the classification and M factors (acute and chronic) proposals.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
09.05.2018	Finland		MemberState	5
Comment received				
There are adequately of toxicity test data available for classification purposes of aquatic hazards of fluxapyroxad. According to studies, the most sensitice trophic group is fish. The acute toxicity LC50 value for common carp (Cyprinus carpio) is between 0.1-1.0 mg/l and the chronic toxicity NOEC value for fathead minnow (Pimephales promelas) is between 0.01-0.1 mg/l. FI CA also supports the conclusions that the substance is neither rapidly degradable nor potentionally bioaccumulative. Based on the available information and the classification criteria, FI CA supports the proposed classification of Aquatic Acute 1, H400 with M-factor of 1 and Aquatic Chronic 1, H410 with M-factor of 1 for fluxapyroxad.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
08.05.2018	Belgium		MemberState	6
Comment received				
BE CA supports the proposed environmental classification for the substance fluxapyroxad : Aquatic Acute 1, H400 (M acute = 1) and Aquatic Chronic 1, H410 (M Chronic=1).				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				