Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

fluopicolide (ISO); 2,6-dichloro-*N*-[3-chloro-5-(trifluoromethyl)-2-pyridylmethyl]benzamide

EC Number: -
CAS Number: 239110-15-7

CLH-O-0000006820-76-01/F

Adopted

11 June 2020

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON FLUOPICOLIDE (ISO); 2,6-DICHLORO-*N*-[3-CHLORO-5-(TRIFLUOROMETHYL)-2-PYRIDYLMETHYL]BENZAMIDE

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.

ECHA accepts no responsibility or liability for the content of this table.

Substance name: fluopicolide (ISO); 2,6-dichloro-*N*-[3-chloro-5-(trifluoromethyl)-2-pyridylmethyl]benzamide
EC number: 607-285-6
CAS number: 239110-15-7
Dossier submitter: Austria

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
10.10.2019	France		Member State	1
Comment received				
FR: FR considers that physical chemical properties of the substance and the environmental hazard assessment should be reported in the CLH report dossier as there are available and assessed in the DAR.				
Dossier Submitter's Response				
It was agreed upfront with ECHA that only the human health hazards will be addressed in this process and that the other areas will be covered within the EU approval renewal process of fluopicolide starting November 2020.				
RAC's response				
Noted. RAC is not able to conclude on these hazards since they had not been assessed in the CLH dossier.				

Date	Country	Organisation	Type of Organisation	Comment number
09.10.2019	Germany		Member State	2
Comment received				
Even if no classification was proposed with regard to the physical and chemical data, at least the most important data relevant to evaluation like water solubility, pH, physical form, particle size or Log(KOW) should be listed.				
Dossier Submitter's Response				
It was agreed upfront with ECHA that only the human health hazards will be addressed in this process and that the other areas will be covered within the EU approval renewal process of fluopicolide starting November 2020.				
RAC's response				
Noted. RAC is not able to conclude on these hazards since they had not been assessed in the CLH dossier.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
09.10.2019	Germany		Member State	3

Comment received

The German CA agrees with the DS that classification for carcinogenicity is not required. This conclusion is mainly based on the occurrence of hepatic neoplasia limited to both sex of one species, the lack of progression to malignancy, and plausible but incomplete mechanistic information that adenoma likely resulted from a rodent specific mechanism.

Nevertheless, two issues should be clarified before a decision is being taken:
- In vivo data: Has the statistical analysis of the rodent carcinogenicity assays been performed according to OECD GD
116 and did it include trend tests?
- Mechanistic data: What is the meaning of "(+)" in table 10-24 as compared to "+" and "-"? While both sexes showed
neoplastic response in vivo, there was a marked difference with regard to BROD induction and labelling between sex
in vitro. This should be discussed also with regard to the reliability of the in vitro model. As required by the
ECHA/WHO/IPCS framework for MoA analysis, alternative mechanisms need to be addressed and excluded. Accordingly,
data on AhR activation and Cyp1a expression should be presented in view of the (moderate) increase in EROD activity,
complemented by a revised conclusion on the activation of the AhR pathway.

Dossier Submitter's Response

Statistical analyses

The statistical analyses used in both the rat combined chronic/carcinogenicity study and in the mouse carcinogenicity study are described in detail in the CLH report and accompanying Annex I document (see pages 156 and 191 of Annex I to the CLH report respectively). Trend tests were included, and the methods used concur with the guidance outlined in section 4 of OECD guideline 116.

Mechanistic data

The symbol (+) in table 10-24 indicates that fluopicolide is a *weak* activator of human PXR (indicated by an increase in CYP3A enzyme activity levels); + and – in the tables indicates a *more substantial* change in enzyme activity. Refer to tables 3.9.4.3-2 to 3.9.4.3-4, 3.9.4.4-2 to 3.9.4.4-4 and 3.9.4.5-2 to 3.9.4.5-5 of Annex I to the CLH report for detailed results.

Sex differences and validity of the *in vitro* assay

Neoplastic responses were seen in both sexes *in vivo*, and PROD/BROD/BQ induction and BrdU labelling were investigated in both male and female mouse hepatocytes *in vitro*. The induction of PROD was similar for both males and females (3-fold at 2 µM and 1.7-fold at 1 µM), whilst the induction of BROD was greater in male than in female hepatocytes (2.5-fold at 2 µM compared with 1.2-fold at 3 µM with the latter being not of statistical significance). However, this apparent sex difference was also observed with phenobarbital (PB), which showed a 7.7-fold increase in males compared with 4.4-fold in females. Furthermore, a sex difference already exists in the solvent control hepatocytes, with BROD induction being 1.8-fold greater in female control hepatocytes than in male controls (24.74% in males compared with 44.9% in females). With regard to DNA synthesis, there was no sex difference observed, with both males and females exhibiting an increase in BrdU labelling (1.7- and 2.3-fold respectively), reflecting the observed increase for

phenobarbital (1.8- and 2.2-fold in males and females), however there was a sex difference in basal proliferation, being 3.8-fold greater in females than in males (0.28 in males compared with 1.07 in females). Although the PB-like response is robust in mice, it is known that the response models poorly in hepatocytes *in vitro* compared with *in vivo.* One feature of the *in vitro* model, as reported by the study director, is that it is common to have a higher induction response in males than females, and equally, females tend to have higher basal rates, so induction in females is often lower as a result. This is the case for fluopicolide. Therefore, the sex differences observed in the *in vitro* study, are likely to be an inherent property of the *in vitro* test system and not a specific effect of fluopicolide.

The reliability of the *in vitro* assay is demonstrated by the expected pattern of enzyme induction/DNA synthesis demonstrated for phenobarbital (marked inductions of PROD and BROD followed by a marked increase in DNA synthesis). Fluopicolide gave similar inductions in PROD/BROD following by an increase in BrdU labelling. Therefore, the assay is considered valid to demonstrate that fluopicolide is able to active CAR *in vitro* and that this activation leads to an increase in DNA synthesis.

It should also be noted that the purpose of the data is to model a PB-like MOA and exclude human relevance, therefore, the data should not be examined in isolation. When taken side-by-side with the CAR/PXR knock out (KO) data it can be seen that there is a clear PB-like response in the Wild Type (WT) hepatocytes compared with the CAR/Pxr KO hepatocytes. This is also supported by the *in vivo* data investigating enzyme induction in mice, which also clearly defines the phenobarbital-like mode of action.

<u>AhR activation</u>
Please refer to Annex 2 within the CLH report for a detailed evaluation of the mode of action according to the WHO/IPCS framework, including the exclusion of other potential MOAs.
AhR enzyme induction can be excluded as a potential mode of action. Fluopicolide did not produce a large increase in P450 Cyp1a EROD activity in the 28-day mechanistic study; only a slight increase of 79% was noted (compared with 1785% and 1143% increases in BROD and PROD respectively). This increase followed a similar pattern to that observed with phenobarbital (PB) in a separate control study, in which PB induced an 83% increase in EROD activity (compared with a 6326% and 1920% increase in BROD and PROD respectively). Furthermore, fluopicolide has been shown to not activate the AhR receptor in a ToxCast assay in which gain of signal (relative to DMSO control) was measured in human HepG2 cell lines, with measurements being taken 24-hours following dosing in a 24-well plate (for further details refer to ToxCast dashboard for fluopicolide).

RAC's response

Noted. RAC agrees with the DS.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON FLUOPICOLIDE (ISO); 2,6-DICHLORO-*N*-[3-CHLORO-5-(TRIFLUOROMETHYL)-2-PYRIDYLMETHYL]BENZAMIDE

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
09.10.2019	Germany		Member State	4
Comment received				
The German CA agrees with the DS that classification for germ cell mutagenicity is not proposed. The PCE/NCE ratio is strongly and significantly altered in the in vivo micronucleus test with i.p. application and therefore bone marrow exposure is considered to be demonstrated.				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted. RAC agrees with the DS.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
07.10.2019	Germany	Bayer AG Crop Science Division	Company-Manufacturer	5
Comment received				
In Annex 1 to the CLH report, a slight delay (3 days) in vaginal opening at the high-dose in the range-finding study was dismissed as non-treatment related on the basis that the time to vaginal opening (36 days) was similar to that of the control in the successive 2-generation study (35 days). Suggesting that the control value (33 days) in the range finding study was unusually low. Since submission of the CLH report historical control data has been made available and are attached which supports this conclusion. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Fluopicolide Commenting Period Historical Control Data.zip				
Dossier Submitter's Response				
New historical control data were submitted by applicant.				
RAC's response				
The submitted historical control data were noted. RAC agreed that no classification for fertility was warranted but concluded that the substance should be classified for development as Repr. 2; H361d.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
07.10.2019	Germany	Bayer AG Crop Science Division	Company-Manufacturer	6
Comment received				
A slight increase in interstitial cell hyperplasia in the testes and Acinar cell atrophy with associated reduced colloid in the prostate were observed in mid- and high-dose males in the 2-year combined rat chronic/carcinogenicity study (see Annex 1 to the CLH report). Since the submission, historical control data relating to this effect has become available and are attached. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Fluopicolide Commenting Period Historical Control Data.zip				
Dossier Submitter's Response				
New historical control data were submitted by applicant.				
RAC's response				
Noted. RAC agrees with the DS.				

Date	Country	Organisation	Type of Organisation	Comment number
09.10.2019	Germany		Member State	7
Comment received				
A classification for STOT RE (Category 2) should be considered. In chapter 10.12.2 of the CLH report the following discussion of mortality was submitted: "In the rabbit developmental toxicity study with doses of 0, 5, 20 or 60 mg/kg bw/day from Day 6-28 of gestation, at the highest tested dose of 60 mg/kg bw/day severe maternal toxicity as evidenced by mortality, marked decreases in body weight gain and food consumption was observed resulting in high incidences of premature delivery. According to the CLP guideline values for classification for STOT RE these could be considered as results triggering STOT RE classification since the increased mortality occurred at a dose below 365 mg/kg bw/day which is the trigger value for STOT RE category 2 adapted to a exposure duration of 23 days. However, mortality was the main sign and no other consistent or significant organ damage was reported." Nevertheless, according to the Guidance on the Application of the CLP Criteria, increased mortality can be sufficient for classification for STOT RE and no further observations of "significant organ damage" are needed. The increased mortality was observed in dose group 60 mg/kg bw/day on days 24, 25 and 29 in a developmental toxicity study in rabbits. It was also observed in the dose-range finding study in rabbits. The potential species sensitivity of the effect/relevance of mortality in two rabbit studies to human health should be discussed.				
Dossier Submitter's Response				
A NOAEL of 20 mg/kg bw/day was set for the rabbit in a developmental toxicity study. In this study, there were no deaths at 5 or 20 mg/kg bw/day. At the high dose of 60 mg/kg bw/day, however, 3 animals were found dead on days 24, 25 and 29 (in the accompanying dose-range finding study 2/4 animals were found dead and 1/4 killed in a moribund state at 100 mg/kg bw/day and 2/4 were found dead and 2/4 killed in a moribund state at 250 mg/kg bw/day). The decedents exhibited bodyweight losses and markedly reduced food consumption from the start of treatment until death. In rats no				

deaths were noted up to and including the highest dose tested of 700 mg/kg bw/day in a developmental toxicity study with a comparable dosing regime, a NOAEL of 60 mg/kg bw/day was set from this rat study based on slight (maximum -12%) reductions in body weight at the high-dose; there were no other indications of general toxicity noted in rats. The large disparity in the observed toxicity from these two comparable studies (and the lack of mortality observed in the rat) demonstrates that the rabbit is far more sensitive to fluopicolide than the rat. Similarly, in other repeated dose studies in rats, mice and dogs no mortality was observed at levels up to 1770 mg/kg bw/day, even after chronic exposure, and the effects seen in rabbits prior to death and in surviving rabbits (large reductions in body weight gain, food consumption and defaecation with clinical signs comprising impairment of motility and consciousness), were not present in any other species. In rabbits, the toxicity of a substance is heavily influenced by the substantial differences in their gastrointestinal (GI) tract, which resembles ruminants and relies substantially on bacteria-mediated digestion in a very large caecum, followed by refection and a second round of absorption. The metabolism of the rabbit is therefore geared towards a vegetarian diet. This is distinct from the rat, which being omnivorous, more resembles the human. Rabbits are generally more sensitive than rats to oral xenobiotic exposure, and disturbances of the gut process is a frequent factor in producing overt toxicity. Therefore, the material differences from humans in the rabbit's GI structure, functioning and homeostasis, which render it more sensitive to disturbance, mean that it is unlikely that the kind of overt toxicity seen in the rabbit would be relevant to humans. Classification for STOT-RE is warranted when repeated exposure to a substance results in significant or severe toxicity at doses that are around or below the assigned reference values. Aside from the mortality (as described above) which was seen only in rabbits and can be considered not relevant to humans, no significant or severe toxicity was observed at relevant doses following administration of fluopicolide. Therefore, classification for STOT-RE is not warranted.
RAC's response
Noted. RAC agrees with the DS.

PUBLIC ATTACHMENTS

1. Fluopicolide Commenting Period Historical Control Data.zip [Please refer to comment No. 5, 6]