Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

2,3,5,6-tetrafluoro-4-(methoxymethyl)benzyl (1*R*,3*R*)-2,2-dimethyl-3-[(1*Z*)-prop-1-en-1-yl] cyclopropanecarboxylate; Epsilon-metofluthrin

EC Number: -
CAS Number: 240494-71-7

CLH-O-0000001412-86-111/F

Adopted
3 June 2016

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2,3,5,6-TETRAFLUORO-4-(METHOXYMETHYL)BENZYL (1R,3R)-2,2-DIMETHYL-3-[(1Z)-PROP-1-EN-1-YL]CYCLOPROPANECARBOXYLATE; EPSILON-METOFLUTHRIN

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: 2,3,5,6-tetrafluoro-4-(methoxymethyl)benzyl (1R,3R)-2,2-dimethyl-3-[(1Z)-prop-1-en-1-yl]cyclopropanecarboxylate; Epsilon-metofluthrin
EC number: -
CAS number: 240494-71-7
Dossier submitter: United Kingdom

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
10.08.2015	United States		Individual	1
Comment received				
I fully agree with the ECHA CLH report as published for public consultation and with the proposed C&L for EU harmonized classification and labelling of the compound.				
Dossier Submitter's Response				
Thank you for your comments.				
RAC's response				
Noted.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
10.08.2015	United States		Individual	2
Comment received				
Based on an extensive array of mechanistic studies, the mode of action for metofluthrin clearly involves activation of constitutive androstane receptor (CAR) leading to an increase in hepatocellular proliferation with consequent ultimate formation of hepatocellular foci, adenomas, and ultimately carcinomas. The types of studies to demonstrate this association have followed closely those that were used to demonstrate this mode of action for the prototypic CAR activator, phenobarbital. Phenobarbital (or its sodium salt) was used as a positive control in these studies involving metofluthrin. There have been several very pivotal experiments demonstrating the relationship of CAR in the events related to metofluthrin in hepatocytes. A pivotal study involved the evaluation of the effect of metofluthrin in rat hepatocytes employing a RNA interference				

technique to lower CAR mRNA levels by CAR-siRNA. Metofluthrin produced a significant induction of CYP2B1 mRNA levels in rat hepatocytes treated with control-siRNA. However, the lowering of CAR mRNA levels by CAR-siRNA resulted in a significant decrease in the magnitude of induction of CYP2B1 mRNA following treatment with metofluthrin, clearly indicating the importance of CAR activation in the mode of action of metofluthrin. Furthermore, treatment of rat hepatocytes in vitro induced CYP2B enzymes and increased proliferation, whereas human hepatocytes showed the enzyme changes but not the increased proliferation response. The ability of the human hepatocytes to respond to a mitogenic stimulus was confirmed using hepatocyte growth factor (HGF).

The relevance of this mode of action to humans has been extensively evaluated. I believe that the most noteworthy demonstration that the mode of action is not relevant to humans is the recent experiment involving administration of phenobarbital to chimeric mice, i.e. mice that have received a transplant of human hepatocytes. The range of replacement indices in chimeric mice used in the present study was 73–90%. Rodent hepatocytes in CD-1 mice and Wistar Hannover rats showed the usual metabolic changes in response to phenobarbital sodium salt and also showed a proliferative response. In contrast, the human hepatocytes in chimeric mice showed the metabolic response but did not show the proliferative response. Since proliferation is an essential key event in the mode of action, its absence in the human cells demonstrates that this mode of action does not translate to humans. This has been corroborated in recent investigations with metofluthrin in a similar chimeric mouse model (Yamada, unpublished observations). Epidemiology studies also strongly support a lack of cancer risk in humans with respect to administration of phenobarbital, even at doses which produce similar blood levels to those which are hepatocarcinogenic in rodents.

With respect to metofluthrin, an examination of alternative modes of action has also been performed. There is no evidence of DNA reactivity (genotoxicity), eliminating that as a potential mode of action. Likewise, there is no evidence of cytotoxicity, either by examination of the histopathology or by an evaluation of liver enzymes in blood associated with hepatocellular damage. Furthermore, there is no activation of PPARα as evidenced by the lack of activation of CYP4A. Also, there is no evidence of iron deposition, no evidence of estrogen-like activity, and no evidence of statin-like effects on HMG (3-hydroxy-3-methyl-glutaryl)-CoA-reductase activity. Of interest, all statins produce high incidences of liver tumors in male and female rats and mice, but an extensive body of epidemiology literature, involving several hundred thousand individuals, shows that statins are not carcinogenic to humans, either to the liver or to any other tissues.

Thus, the mode of action for metofluthrin has been demonstrated to involve activation of CAR. It does not involve any of the other modes of action that have been identified for liver hepatocellular carcinogenesis, and involves a mode of action that is not relevant to human cancer risk. Based on these observations, I strongly support the conclusion of the UK Competent Authority (Chemicals Regulation Directorate) to not classify metofluthrin with regard to cancer.

My detailed discussion is in the attached document.

ECHA comment: the following attachment was provided with this comment:

1. Comments on the mode of action for metofluthrin-induced rat hepatocellular tumors and an evaluation of their human relevance

Dossier Submitter's Response
Thank you for your comments and additional information. We have no further comments.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
10.08.2015	United Kingdom		Individual	3
Comment received				
Metofluthrin produces liver tumours in rats by an established mode of action which is not relevant for humans. *ECHA comment: the following attachment was submitted with this comment:* 2. Expert Statement on Metofluthrin (CLH Report, Version 2 of May 2015). Professor Brian G. Lake, Centre for Toxicology, University of Surrey, Guildford, Surrey, UK				
Dossier Submitter's Response				
Thank you for your comments.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
06.08.2015	United States	Exponent International, for Sumitomo Chemical (UK)	BehalfOfAnOrganisation	4
Comment received				
Sumitomo Chemical strongly supports the CLH recommendation of no classification for carcinogenicity. A Mode of Action via CAR activation is clearly shown, and is supported by a Human Relevance analysis (Yamada 2012). No classification is consistent with RAC Opinion precedents for Sulfoxaflor (2014) and Fenpyrazamine (2012).				
Dossier Submitter's Response				
Thank you for your comments.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
13.08.2015	Germany		Member State	5
Comment received				
Pages 39 - 54 In contrast to the UK proposal, classification for carcinogenicity is considered necessary. The test substance clearly produced a marked, statistically significant (at top dose level of 78 or 96 mg/kg bw/day) and at least in the two upper dose levels dose-related increase in liver adenoma and carcinoma in both male and female rats. It could be shown that these tumours were likely related to CAR activation even though it is surprising that no such response was noted in mice. According to the "Guidance on the application of CLP				

criteria", CAR-related tumours are not explicitly stated as "not relevant for humans". However, the available data suggest that classification as cat. 2 carcinogen (H351) would be more appropriate than grouping into cat. 1.
Dossier Submitter's Response
Thank you for your comments. We have no further comments at this stage, our full rationale for the proposal is provided in the CLH report.
RAC's response
RAC notes that liver tumours were not induced in mice. This seems inconsistent, as mice generally appear more susceptible than rats to liver tumour formation by CAR activators. However, for some CYP2B enzyme inducers which appear to have a similar MoA for liver tumour formation to PB, such as pyrethrins and momfluorothrin, liver tumours have been observed in rats but not in mice (Elcombe *et al*., 2014). Besides, comparison of the magnitude of effect on relative liver weight in the 90-day studies also showed that effects in mice were less pronounced than in rats. Ttoxicokinetic differences may explain a difference in sensitivity between species. All in all, RAC considers that the available evidence supports that liver tumours in the rat are induced as a consequence of CAR inductionby metofluthrin. In contrast to rat hepatocytes, it has been shown that cell proliferation in response to CAR activation by metofluthrin is not observed in human hepatocytes. This step is considered essential for liver tumour formation and therefore the liver tumours observed in rats are of little relevance for humans.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
10.08.2015	United Kingdom		Individual	6
Comment received				
Metofluthrin is not genotoxic.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
10.08.2015	United States		Individual	7
Comment received				
I fully agree with the proposed classification.				
Dossier Submitter's Response				
Thank you for your comments.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
06.08.2015	United States	Exponent International, for Sumitomo Chemical (UK)	BehalfOfAnOrganisation	8

Comment received

See Exponent document 0800265.uk0 – 4028, attached.
In outline: Sumitomo Chemical disagrees the proposal for oral Acute Tox 3 based on a neurotoxicity study using corn oil as a dosing vehicle. Corn oil is not a substance in which metofluthrin will be supplied and does not comply with Article 8(6) of the CLP Regulation (1272/2008); it is not an appropriate vehicle for quantitative estimation of the ATE.
P9, 2.2 Short summary of the proposal: mortality is selectively presented, and not representative. Necessity to harvest neurotoxicity tissue samples affected the decision to sacrifice animals, so not the same as for an acute toxicity study.
P19, 4.2.1.2 Acute toxicity- inhalation: No information is stated. Sumitomo Chemical takes the view that a detailed description of time course and symptoms observed in the inhalation study (Yoshihito, 2002) show that inhalation toxicity (both symptoms and mortality) occurs more rapidly than by dosing orally in corn oil; although signs of toxicity are qualitatively similar to those obtained by oral dosing. These findings of absorption and the Tmax being more rapid than by oral absorption contradict later speculation on toxicokinetics due to corn oil.
P20, 4.2.3; Statement on "vehicle dependant differences in toxicokinetics" is speculative. Appropriate consideration of the inhalation study data does not support this speculation.

ECHA comment: the following attachments were submitted with this comment:

3. Epsilon-Metofluthrin: Comments on the CLH Report
4. Metofluthrin: Acute Oral Toxicity Classification

Dossier Submitter's Response

Thank you for your comments. We note the discrepancy between the results of the standard (OECD 401) acute study and the neurotoxicity study and have acknowledged this in the CLH report. However, corn oil is a standard dosing vehicle for oral gavage toxicology studies, and unless a sound case can be made that the use of corn oil is not appropriate for a particular substance, results of studies using corn oil should be considered relevant for hazard identification purposes.

We note, your comments regarding the sacrifice of the animals. However, the study summary notes that that 4 animals were sacrificed in moribund condition prior to the scheduled sacrifice. In our opinion, this implies that the animals were experiencing significant distress and the decision to euthanize these animals appears to have been taken on animal welfare grounds. This would to be consistent with EU law on the protection of laboratory animals (Directive 2010/63) and with the OECD guidance on Humane End-Points (OECD GD 19).

Therefore, we remain of the opinion that this study should be taken into consideration in the classification. However, if a classification for acute toxicity is not considered appropriate, a classification for STOT-SE 1 or 2 could be applied. See further comments in response to comment 11.

RAC's response
RAC agrees with the DS that the administration of metofluthrin in corn oil is appropriate for classification purposes. The slightly earlier onset of clinical signs and/or mortality after the start of exposure by inhalation, in comparison to gavage in corn oil, may also reflect differences in the relative levels of exposure. This may have an impact on the time needed to reach systemic effective levels. However, comparisons of inhalation and oral data support the LD_{50} by gavage in corn oil being lower than 300 mg/kg. Therefore, the criteria for classification as Acute Tox 3 – H301 are met.

Date	Country	Organisation	Type of Organisation	Comment number
31.07.2015	France		Member State	9
Comment received				
FR-CA has the opinion that a classification Acute Tox 3; H301 is not justified. Indeed, in a guideline OECD 401 study, the substance is administered without vehicle and does not induce 50% of mortality and thus no classification is warranted. In the acute oral neurotoxicity study, as eMSCA mentioned, this is the vehicle, corn oil, which can increase the toxicity leading to the proposed classification of the substance. Moreover, this effect is not mentioned in other available studies for similar doses. Indeed, there is no death in an acute neurotoxicity screening study and in a non-GLP study, at this same dose. Finally, at similar doses in the repeated toxicity studies, mortality is not mentioned. Based on these arguments, FR-CA proposes to not classify the substance.				
Dossier Submitter's Response				
Thank you for your comments. Please see our response to comment number 8.				
RAC's response				
Considering the results obtained in the different experiments of the acute neurotoxicity study together, 20% of mortality was observed at 100 mg/kg in rats exposed through gavage in corn oil. Similarly, 20% of mortality was observed in mice in a sighting micronucleus test with a similar mode of administration. Such mortality was not observed at similar dose levels in repeated dose toxicity studies but in these studies the substance was administered in the diet. In prenatal studies, gavage in corn oil was also used and mortality was observed at 30 mg/kg, which is consistent with other available acute data. This further supports the mode of administration significantly influencing the dose levels that induce toxic effects. RAC agrees with the DS that the administration of metofluthrin in corn oil is appropriate for classification purposes.				

Date	Country	Organisation	Type of Organisation	Comment number
13.08.2015	Germany		Member State	10
Comment received				
Pages 18 – 21 The UK proposals (Acute Tox 3, H301 and Acute Tox 4, H332) for acute oral and inhalative toxicity are supported.				
Dossier Submitter's Response				
Thank you for your comments.				

RAC's response
Noted.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
06.08.2015	United States	Exponent International, for Sumitomo Chemical (UK)	BehalfOfAnOrganisation	11

Comment received
Sumitomo Chemical supports the CLH recommendation of no classification for STOT-SE. Findings of neurotoxicity were seen only at dose levels associated with mortality. Data for oral toxicity using a corn oil vehicle are not appropriate (in line with argument for the acute toxicity ATE) to compare with the cut-off for STOT-SE.
Dossier Submitter's Response
Thank you for your comments. Clinical signs of toxicity, consistent with neurotoxicity, were observed in the acute oral and inhalation exposure studies, as well as the acute neurotoxicity studies. In the acute oral study, at 2000 mg/kg the following effects were observed; tremors, twitches, tachypnoea, prostrate, loss of righting reflex, clonic convulsions, tonic extensor convulsions and hyperpnoea. At the lower doses of 1000 and 1500 mg/kg, tremors and tiptoe gait were observed. All effects were resolved by day 3. In the acute neurotoxicity study, at 100 mg/kg the following effects were observed; twitches (7/10 m and 4/10 f), whole body intermittent and/or continuous tremors (8/10m and 8/10f), clonic convulsion (1/10m) and tonic extensor convulsions (1/10f). In the acute inhalation study from 0.5 mg/l tail tremors were observed during exposure and tremors, hypersensitivity, ataxic gait, tiptoe gait and clonic convulsions were reported post exposure. All signs had resolved one day after cessation of exposure. In the case of the acute neurotoxicity study, these effects were observed at a dose causing mortality (3 animals died and 4 sacrificed in a moribund condition). In the acute inhalation study, 1/5 females died following exposure to 1 mg/l, whereas 5/5 females and 5/5 males died at a dose of 2 mg/l. As noted in the response to comment number 8, if classification for acute toxicity is not supported, then classification for STOT SE (neurotoxicity) could be applied, given the consistent clinical signs of neurotoxicity noted in all studies. Also, it is noted in the biocide assessment that metofluthrin is a synthetic pyrethroid, which interferes with nerve function in a manner that is identical to the standard pyrethroid mode of action (i.e., blocking of the sodium channels).
RAC's response
In the acute toxicity study by inhalation, serious signs of neurotoxicity are described at all doses including the lowest dose of 0.5 mg/L, which does not induce lethality. In addition, tremor is observed in the 28-day inhalation study (rat) at 0.2 mg/L and is considered an acute effect, whereas mortality at this dose is attributed to multiple exposure.

Altogether, these data support classification as STOT SE 1 (nervous system).

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
06.08.2015	United States	Exponent International, for Sumitomo Chemical (UK)	BehalfOfAnOrganisation	12
Comment received				
p24, 4.3.2 The reasoning for the conclusion "not considered to be a manifestation of acute toxicity" is not explained. There is a clear element of acute toxicity: at least 4 of the decedents died within 1 hour of the end of a daily exposure period; symptoms were most marked immediately after exposure and resolved by morning of the following day. If these mortalities can be concluded to be a manifestation of acute toxicity, classification for STOT-RE is not appropriate.				
Dossier Submitter's Response				
7/10 males and 3/10 females died in the 28-day inhalation study. Whilst it is noted that a number of deaths occurred within 1 hour of the daily exposure period, the deaths were spread throughout the study. The 3 females died on days 3, 4 and 5 respectively and the male deaths were distributed throughout the study with 3 on day 4 and 1 on each of days 9, 19, 25 and 27 respectively. Therefore, on balance, we remain of the opinion that this is not entirely explained as a manifestation of acute toxicity and classification for STOT-RE is appropriate.				
RAC's response				
The mortality observed in the 28-day study by inhalation occurs at lower doses than in the acute toxicity study and is distributed throughout the study. RAC agrees thatclassification as STOT RE 2 is justified on this basis.				

Date	Country	Organisation	Type of Organisation	Comment number
13.08.2015	Germany		Member State	13
Comment received				
Pages 29 – 38 The UK proposal for classification of Epsilon-metofluthrin as STOT RE 2 (Inhalation), H373 is supported because of mortality and tremors in a subacute inhalation study.				
Dossier Submitter's Response				
Thank you for your comments.				
RAC's response				
Noted.				

NON-CONFIDENTIAL ATTACHMENTS:

1. **Comments on the mode of action for metofluthrin-induced rat hepatocellular tumors and an evaluation of their human relevance** – provided by an individual from the United States on 10 August 2015. *(please refer to comment 2)*

2. **Expert Statement on Metofluthrin (CLH Report, Version 2 of May 2015). Professor Brian G. Lake, Centre for Toxicology, University of Surrey, Guildford, Surrey, UK –** submitted by an individual from the United Kingdom on 10 August 2015. *(please refer to comment 3)*

3. **Epsilon-Metofluthrin: Comments on the CLH Report** – submitted by Exponent International, for Sumitomo Chemical (UK) on 6 August 2015. *(please refer to comment 8)*

4. **Metofluthrin: Acute Oral Toxicity Classification** - submitted by Exponent International, for Sumitomo Chemical (UK) on 6 August 2015. *(please refer to comment 8)*