Committee for Risk Assessment

RAC

Annex 3
Records
of the targeted public consultation on the carcinogenicity 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

ANNEX 3 – RECORDS OF THE TARGETED PUBLIC CONSULTATION ON THE CARCINOGENICITY 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

The proposal for the harmonised classification and labelling (CLH) of epsilon-metoflutrin was submitted by the United Kingdom in June 2015; it was subject to public consultation from 30 June 2015 until 14 August 2015. The comments received by that date are compiled in Annex 2 to this opinion.

After the closure of the public consultation, the Committee for Risk Assessment (RAC) received a further study from Industry, which is "**The effect of metofluthrin and momfluorothrin on cell proliferation of human hepatocytes in chimeric mice"** (Tomoya Yamada 2015). The study was not flagged for submission during public consultation by Industry, but was mentioned by two individuals. In order to be able to take the new study into account, it was subject to a two week targeted consultation, starting on **30 March 2016** and ending on **13 April 2016**. The comments received are compiled in this annex.

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Substance name: **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**

Date	Country / Person / Organisation / MSCA	Comment	RAC response to comment
11/04/2016	**Sweden (MS CA)**	The Swedish CA welcomes the opportunity to comment on this novel type of study. To our knowledge, this type of study has never been used in the EU as a basis to decide how to classify chemicals. It is an interesting system that avoids some problems (eg. in relation to interpreting alternative in vitro hepatocyte data) but introduces many other issues; • How will the rodent environment (e.g. rodent hormones) affect the human liver cells? Will human hepatocytes act differently in a rodent environment than in a human environment? • The mouse strain is described as immuno-deficient, with a liver disease. How will this affect the human hepatocytes? In response to metofluthrin, there is no increase in human CYP2B6 mRNA (≤1.4-fold increase) and only a 3-4-fold induction of mouse Cyp2b10 mRNA. Does this indicate that CAR is not activated? Or perhaps that the model does not work?	The limitations of the study raised by the Swedish CA are noted. RAC agrees that this specific experimental design raises uncertainties: immaturity of human hepatocytes transfected, specificities of the strain unknown, possible influence of the murine environment while mice is not sensitive to metofluthrin carcinogenicity. It is noted that similar results in this specific experimental design has been observed with sodium phenobarbital (Yamada 2014[1]). In addition, a weak but significant increase in human CYP2B6 mRNA is observed in 2/3 experiments, which is in line with the modest induction of CYP observed *in vitro* in human hepatocytes. Overall, this study is therefore considered consistent with the overall database.

[1] Yamada T *et al*. Human hepatocytes support the hypertrophic but not the hyperplastic response to the murine nongenotoxic hepatocarcinogen sodium phenobarbital in an in vivo study using a chimeric mouse with humanized liver. Toxicol Sci. 2014 Nov;142(1):137-57. doi: 10.1093/toxsci/kfu173. Epub 2014 Aug 21.

		• It would be interesting to know if/how this model has been validated. Has there been any studies on typical model substances such as phenobarbital, or other substances (e.g. ligands of other receptors such as the Ah-receptor)? • The size of the liver in this strain is twice as big (in relation to body weight) as other normal mouse strains (12% of bw in the SCID mice vs 6% of bw in other mice (Jones et al, 1992)). How will this affect the mice? How will this affect the metabolism of chemicals, and in this case the metabolism of epsilon-metofluthrin? How will this affect the toxicity of chemicals (if toxicity (CAR-activation) is dependent on metabolism then toxicity will be increased, if metabolism detoxifies then the toxicity (CAR-activation) will be decreased). In addition to having more metabolites, there might also be other metabolites formed from the chemical in the SCID mice than in normal mice. • The human hepatocytes come from children, but once in adult mice, do the human hepatocytes still have child characteristics or do they mature into adult human hepatocytes, and how does this affect e.g. the development of the cell machinery (receptors, metabolism, etc.)? • In studying cell proliferation and CYP induction, hEGF is used as a control. hEGF is a ligand for a cell membrane receptor, whereas CAR is a nuclear receptor. To our knowledge these receptors don't mediate cell proliferation through the same pathways. We agree that the use of hEGF shows that the cells can proliferate, but does it prove that CAR-mediated proliferation in human hepatocytes can be excluded under these test conditions?	Having in mind the inherent limitations of this study, it is considered to provide an additional element of relevance in the weight of evidence pointing toward the absence of proliferation of human hepatocytes in response to metofluthrin CAR-induction.

		• A technical detail is that we wonder how easy it is to distinguish human from mouse hepatocytes, for instance when measuring cell proliferation using BrDU-labelling? To summarize, we have a lot of questions about the reliability of this model, and look forward to the response to our questions. At present, we are hesitant to draw any conclusions as to the carcinogenic MoA of epsilon-metofluthrin based on this study. Finally, we have noted that the model requires use of human hepatocytes from young children, in this case 2-5 years of age. The project has obviously an ethical approval from the PhoenixBio Ethics Board, but we feel uneasy about using child hepatocytes as we wonder if the parents knew that the donated hepatocytes would be used for toxicological testing of pesticides. **Reference** Jones et al, 1992, • (http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1497&context=animalscifacpub&sei-redir=1&referer=http%3A%2F%2Fwww.bing.com%2Fsearch%3Fq%3Dliver%2Bbody%2Bweight%2Bratio%2Bmouse%26src%3DIE-SearchBox%26FORM%3DIENTTR%26conversationid%3D#search=%22liver%20body%20weight%20ratio%20mouse%22)	