Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

pethoxamid (ISO); 2-chloro-*N*-(2-ethoxyethyl)-*N*-(2-methyl-1-phenylprop-1-enyl)acetamide

EC Number: -
CAS Number: 106700-29-2

CLH-O-0000007269-65-01/F

Adopted

16 March 2023

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON PETHOXAMID (ISO); 2-CHLORO-N-(2-ETHOXYETHYL)-N-(2-METHYL-1-PHENYLPROP-1-ENYL)ACETAMIDE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during 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 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 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. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Substance name: pethoxamid (ISO); 2-chloro-*N*-(2-ethoxyethyl)-*N*-(2-methyl-1-phenylprop-1-enyl)acetamide
EC number: -
CAS number: 106700-29-2
Dossier submitter: Austria

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
03.06.2022	Germany		MemberState	1
Comment received				
The proposed classification is supported.				
Dossier Submitter's Response				
Thank you.				
RAC's response				
RAC has taken note of your comment.				

Date	Country	Organisation	Type of Organisation	Comment number
09.06.2022	United States of America	FMC	Company-Manufacturer	2
Comment received				
FMC submits the following comments for consideration by the Risk Assessment Committee (RAC) regarding the proposed Harmonised Classification and Labelling of pethoxamid. FMC agrees with the Dossier Submitter (DS) that it is appropriate to classify pethoxamid for Acute Tox, Cat. 4 (ATE = 983 mg/kg bw) (H302), Skin Sens., Cat. 1A (H317), Aquatic Acute 1 (M-factor = 100) (H400), and Aquatic Chronic 1 (M-factor = 10) (H410). FMC agrees with the DS that pethoxamid does not meet the classification criteria for genotoxicity, carcinogenicity, reproductive toxicity, specific target organ toxicity – single and repeated exposure, and aspiration hazard. A document containing FMC's full comments is attached to this submission. ECHA note – An attachment was submitted with the comment above. Refer to public				

attachment Pethoxamid, FMC comments on CLH dossier, 9 June 2022.pdf
Dossier Submitter's Response
Noted.Thank you.
RAC's response
RAC has taken note of your comments.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
10.06.2022	Switzerland	Federal Food Safety and Veterinary Office	National Authority	3

Comment received

CLH report, section 10.9, pages 30-45 and annex, section 3.9, pages 35-97:
Regarding the chronic toxicity and carcinogenicity study performed in the rat, it is stated on page 31 of the CLH report that the only statistically significant evidence of tumourigenicity was a higher incidence of thyroid follicular cell adenoma in males at a dose of 1600 ppm. It is concluded that the mode of action is not relevant to humans. On page 42 of the annex to the CLH report, it is specified that due to differences in thyroid physiology between rodents and humans, thyroid tumours in rodents consequent to the phenobarbitone-like mode of action are not considered relevant to humans. Along the same lines, the relevance of hepatocellular adenoma observed in the mouse carcinogenicity study at a dose of 5000 ppm is dismissed on page 32 of the CLH report based on a phenobarbitone-like mode of action for pethoxamid.

This assessment raises two concerns:

1) According to a recent article, the mechanisms by which phenobarbital causes tumour promotion or inhibition are still not fully understood (Braeuning and Schwarz (2016), Archives of Toxicology 90:1525). In an earlier article, Braeuning et al. point out that several large epidemiological studies on epileptics who received prolonged treatment with phenobarbital have been conducted which, on the one hand, have provided little evidence that phenobarbital is carcinogenic to humans, but cannot, on the other hand, rule out this possibility (Braeuning et al. (2014), Toxicological Sciences 140:259). Similarly, La Vecchia and Negri deemed that epidemiological data on the specific role of phenobarbital in human liver cancer are limited (La Vecchia and Negri (2014), European Journal of Cancer Prevention 23:1). The International Agency for Research on Cancer concluded in its monograph that there is inadequate evidence in humans for the carcinogenicity of phenobarbital, and hence, deemed that phenobarbital is possibly carcinogenic to humans based on the available data from animal experiments (IARC monographs volume 79). Overall, the current body of literature does not appear to permit to unambiguously conclude that the carcinogenic effects of phenobarbital in rodents are not relevant to humans.

2) If pethoxamid displayed a phenobarbital-like mode of action, one hallmark would be the induction of CYP enzymes in rodent liver, in particular CYP2B subfamily enzymes (Elcombe et al. (2014), Critical Reviews in Toxicology 44:64). However, the results of the quantitative PCR analyses presented on page 94 of the annex to the CLH report reveal no difference with regard to CYP2b10 expression levels in primary mouse hepatocytes treated with the vehicle control and those treated with pethoxamid when the variability of the measurements is taken into account. This holds also true for CYP3a11 expression levels in primary mouse hepatocytes of the 1, 10 and 20 µM treatment groups, albeit a

difference is observed upon treatment with 3 µM pethoxamid. In a further study summarised on pages 65-68 of the annex to the CLH report, the effects of pethoxamid on liver microsomal cytochrome P450 enzyme activity and mRNA levels were assessed in male mice. Treatment with pethoxamid led to an increase of CYP2b10, CYP3a11 and CYP4a10 mRNA levels in liver tissue of up to 115-fold (CYP2b10, 5000 ppm). In turn, a >2-fold increase in enzyme activity was solely observed with regard to CYP3a11/13 at a dose of 400 ppm. No increase of CYP3a11/13 activity was observed at a dose of 5000 ppm. CYP1a1/2, CYP2b10 and CYP4a10/12 did not display increased activity at any of the dose levels assessed, whereas treatment with the reference substance phenobarbital caused a 2.94-, 3.44-fold and 3.28-fold increase in CYP2b10 activity. Overall, even though the data reveal some key characteristics of a phenobarbital-like mode of action, the above-mentioned data illustrate that not all hallmarks are apparent. It should be noted that the sample size is not specified in section 3.9.4.5 of the annex to the CLH report.

In light of the uncertainty regarding the relevance of carcinogenic effects induced by phenobarbital in animal experiments for humans and with respect to the phenobarbital-like mode of action of pethoxamid, we think the relevance of the tumours observed in the carcinogenicity studies should not be dismissed. Regarding the thyroid effects observed in rats in particular, we are of the opinion that it should be presumed that chemicals producing rodent thyroid tumours may pose a carcinogenic hazard for the human thyroid (EPA/630/R-97/002). Without chemical-specific information, humans should be considered as sensitive to carcinogenic effects as are rodents (EPA/630/R-97/002, Hill et al. (1998), Environmental Health Perspectives 106:447). Regarding a possible mode of action, genotoxicity should be considered, as bone marrow exposure in the in vivo micronucleus test in mice cannot be inferred from the ADME data that was generated in rats (see comments on mutagenicity).

Dossier Submitter's Response

Thank you for the comment.
Ad 1) To our knowledge evidence for a clear species difference of CAR-dependent liver cancer is based on epidemiological and clinical studies mainly on the lack of an association between the clinical use of phenobarbital (PB) and the occurrence of liver cancer in humans. This is contrary to the findings in rodents. In addition to *in vitro* experiments (see explanation below) furthermore, experiments using humanized mice further substantiate this species difference (1). However, we acknowledge the arguments made by Bräuning and colleagues that there are data in the scientific literature that challenge this viewpoint. We also accept, that the reason for this species difference remains uncertain. There are indications in the literature that phenobarbital treatment in human primary hepatocytes increase CYP2B mRNA levels but not replicative DNA synthesis or cell proliferations (2,3) whereas rat hepatocyte proliferation was enhanced. In addition, there is evidence human liver chimeric mice where PB did not promote replicative DNA synthesis and proliferation of hepatocytes whilst CYP2B mRNA levels were found to be increased during PB treatment (4).
References:

(1) Yamada T. Application of humanized mice to toxicology studies: Evaluation of the human relevance of the mode of action for rodent liver tumor formation by activators of the constitutive androstane receptor (CAR). J Toxicol Pathol. 2021 Oct;34(4):283-297. doi: 10.1293/tox.2021-0027. Epub 2021 Jun 27. PMID: 34629731; PMCID: PMC8484926.
(2) Parzefall W, Erber E, Sedivy R, Schulte-Hermann R. Testing for induction of DNA synthesis in human hepatocyte primary cultures by rat liver tumor promoters. Cancer Res. 1991 Feb 15;51(4):1143-7. PMID: 1705168.
(3) Yu Okuda et al. Evaluation of the human relevance of the constitutive

androstane receptor-mediated mode of action for rat hepatocellular tumor formation by the synthetic pyrethroid momfluorothrin,The Journal of Toxicological Sciences,2017; https://doi.org/10.2131/jts.42.773

(4) Shizu R, Yoshinari K. Nuclear receptor CAR-mediated liver cancer and its species differences. Expert Opin Drug Metab Toxicol. 2020 Apr;16(4):343-351. doi: 10.1080/17425255.2020.1746268. Epub 2020 Mar 26. PMID: 32202166.

Ad 2)

- Concerning the 2018TOX-PXA4482 Study (2019)- the induction of CYP2B10 and CYP3A11 in primary male mouse hepatocytes is indeed much weaker as compared to PB. However, the concentration of PB is also up to 50 times higher. In addition, for human risk assessment of rodent liver tumor formation produced by non-genotoxic CAR activators it is also very important to evaluate whether CAR activators can stimulate RDS in human hepatocytes. And this has been clearly demonstrated to be not the case. Nevertheless, we fully agree on the remaining uncertainties since no MoA can be demonstrated and the enzyme induction is not always completely inline with that of PB.
- Concerning the sample size of the study summarized in section 3.9.4.5, eight samples per group have been used.
- We agree that there are uncertainties related to the PB-like MoA of pethoxamide. Nevertheless, since CAR and PXR receptors regulate overlapping sets of genes and PXR activation can also produce CYP2B induction the output of such in vitro studies might not always paint a clear pattern (5).

References:

(5) Tomoya Yamada, Samuel M. Cohen & Brian G. Lake (2021) Critical evaluation of the human relevance of the mode of action for rodent liver tumor formation by activators of the constitutive androstane receptor (CAR), Critical Reviews in Toxicology, 51:5, 373-394, DOI: 10.1080/10408444.2021.1939654

RAC's response

RAC has taken note of your comments.

Date	Country	Organisation	Type of Organisation	Comment number
03.06.2022	Germany		MemberState	4

Comment received

Thyroid adenomas in male rats

The incidence of thyroid adenomas was statistically significant (trend-test) increased in male rats. Various mechanistic studies on the mode of action have been performed to elucidate, whether this effect is relevant to humans according to the Bradford Hill criteria. It can be seen from the CLH-report (including the annex), that the key events 1, 2, 4 und 5 (listed on p. 43) have been confirmed by mechanistic studies. Key event 3 (Decreased (initially) serum T4 / T3) is not so clearly demonstrated. Two mechanistic studies (Anonymous (2000) 94 PXA, Anonymous (2016) 1538 PXA) did not detect a decrease of T4 / T3. The study from Anonymous (2020) 2018TOXPXA4560 is described in the CLP-report (p. 37) to show no effect on total T3 or rT3, but a "Time-dependent decrease in total T4 relative to pre-treatment values during the first 29 day". It can be taken from table 3.9.4-17 of the annex I (p. 79) that a slight decrease of T4 occurred comparing the values of the dosed groups with the corresponding pre-treatment values (-3d), unfortunately a statistical analysis was not performed on this. However, this decrease is not clear comparing the values of the dosed groups with those of the control group, what demonstrates a certain variability of the T4-concentration. Comparing the 5000 ppm-group with the control group, there is even a statistically significant increase of T4 at d15,

d29, d57 and d89. Apart from these uncertainties about key event 3 it is overall acknowledged that in light of the Guidance (p. 387) the slight increase of thyroid adenomas at the top dose in male rats is not sufficient to derive a classification.

Liver adenomas in male mice

The incidence of liver adenomas was statistically significant increased in male mice at the highest dose of 982 mg/kg bw/d. Mechanistic studies on the mode of action have been performed to clarify, whether this effect is relevant to humans according to the Bradford Hill criteria. It can be taken from the CLH-report (including the annex), that the key events 1 and 2 (as listed on p. 46) were confirmed. With respect to Anonymous; (2019) 2018TOXPXA4482) it is noted, that the increase in Cyp2b10 or Cyp3a11 was quite weak and not statistically significant. Thus, pethoxamid is only a weak inducer of CAR and/or PXR in vitro. Importantly, it could be demonstrated, that pethoxamid induces replicative DNA synthesis in mouse hepatocytes, but not in human hepatocytes. This might explain the incidence at the top dose (34/50, control: 19/50) as consequence of the amplification of the background incidence by increased cell proliferation, which is limited to mice. It seems, that increased preneoplastic foci (key event 3) were not detected, but overall it is supported, that a classification is not justified.

Dossier Submitter's Response

Thank you for the comment. We agree with these arguments. As also noted in comment number three, the increase in the CYP2 and CYP3 was quite weak and there might be several reasons for that, including lower affinity to the receptor. However, this does not exclude pethoxamide as being a CAR/PXR activator. Also, the lack of RDS in human hepatocytes is a strong indicator for a PB-mode of action.

RAC's response

RAC has taken note of your comments.

Date	Country	Organisation	Type of Organisation	Comment number
09.06.2022	United States of America	FMC	Company-Manufacturer	5

Comment received

FMC agrees with the DS's proposal to not classify pethoxamid for carcinogenicity, based on the lack of human relevance of liver tumours observed in mice and thyroid tumours observed in rats following treatment with pethoxamid.

Recent additional mechanistic of action (MOA) data has been generated that includes a side-by-side assessment of phenobarbital, a model rodent liver and thyroid tumour inducer, to ensure a direct comparison to pethoxamid. These data strongly demonstrate that the hepatocellular adenomas/carcinomas and thyroid follicular cell adenomas observed in rodents treated with high doses of pethoxamid are not relevant to humans.

Pethoxamid has been demonstrated to be a weak inducer of CAR (Constitutive Androstane Receptor) in mouse hepatocyte cultures; and in an in vitro species comparison assay, pethoxamid induced cell proliferation in mouse hepatocytes but not in human hepatocytes. The available data with pethoxamid supports the following MOA where the molecular initiating event is activation of CAR followed by increased cell proliferation leading to increased preneoplastic foci and ultimately increased hepatocellular adenomas. Based on the difference in biological response in humans and

rodents to CAR activation, any hepatocellular adenomas developed in mice through activation of these nuclear receptors by pethoxamid in mice, are not of relevance to humans. Therefore, it can be concluded that pethoxamid does not pose a hepatic carcinogenic hazard to humans.

Based on a recent thyroid MOA study with pethoxamid in rats, it can be concluded that liver enzyme induction, leading to increased hepatic T4 glucuronidation and clearance, elicited a feedback response on the thyroid via an increase in TSH, resulting in associated thyroid follicular cell hypertrophy and hyperplasia due to functional compensation by the thyroid. The results with pethoxamid were consistent with those of phenobarbital, which was included in the study. In a separate biliary excretion study, increased clearance of T4-glucuronide by the liver was demonstrated in pethoxamid-treated rats, consistent with that of phenobarbital, which was included in the study. As noted in the ECHA Guidance on the Application of the CLP Criteria (July 2017), some rodent tumours are not considered relevant for humans, including thyroid tumours mediated by UDP glucuronyltransferase induction. Thus, a concordant and highly plausible MOA has been established for pethoxamid-induced thyroid follicular cell adenomas in rats, a MOA that is not relevant to humans. Therefore, it can be concluded that pethoxamid does not pose a thyroid carcinogenic hazard to humans.

Thus, FMC agrees that pethoxamid does not meet the classification criteria for carcinogenicity (conclusive but not sufficient for classification).

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Pethoxamid, FMC comments on CLH dossier, 9 June 2022.pdf

Dossier Submitter's Response

Noted. Thank you.

RAC's response

RAC has taken note of your comments.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
10.06.2022	Switzerland	Federal Food Safety and Veterinary Office	National Authority	6

Comment received

CLH report, section 10.8, pages 23-26 and annex, section 3.8.1.3, page 26:
According to the current version of the OECD guideline #473 adopted on 29 July 2016, a test chemical is considered to be clearly positive if (…):
a) at least one of the test concentrations exhibits a statistically significant increase compared with the concurrent negative control,
b) the increase is dose-related when evaluated with an appropriate trend test,
c) any of the results are outside the distribution of the historical negative control data (e.g. Poisson-based 95% control limits)
Based on the information given in the annex to the CLH report, it is unclear whether a trend test was performed and/or the results were compared to the distribution of the historical negative control data. As a consequence, it is not clear whether or not the conclusion that pethoxamid demonstrates clastogenic activity both in the absence and presence of S9 mix is based to the assessment criteria laid out in the latest version of the above-mentioned OECD guidance.

CLH report, section 10.8, page 29 and annex, section 3.8.2.1, page 32:
In the context of the in vivo micronucleus test, the dossier submitter argues on page 29 of the CLH report that exposure of the bone marrow to the test substance was demonstrated in an ADME study in rats, where pethoxamid could be detected in bone tissue after a single oral dose of 300 mg/kg bw. As the in vivo micronucleus test was carried out in mice, this statement disregards possible interspecies differences. Differences between species are, however, frequently observed and often unpredictable, and may arise from different ADME profiles (Toutain et al. (2010), Handb. Exp. Pharmacol. 199:19). According to the OECD guideline #473 (29 July 2016), evidence of bone marrow exposure may also include a depression of the immature to mature erythrocyte ratio or measurement of the plasma or blood levels of the test substance. However, on page 33 of the annex to the CLH report it is stated that no effect was observed on the proportion of immature erythrocytes, indicating no bone marrow toxicity. Overall, the available data do not appear to permit to conclude that bone marrow exposure occurred, and hence, clastogenic effects observed in vitro may not have occurred in vivo because the bone marrow of mice was not (sufficiently) exposed to the test substance.

Dossier Submitter's Response

Thank you for the comment.
Within the study report of 76 PXA (section 3.8.1.3) only a Fisher's test was performed to compare each treatment group with solvent control value. No trend test was reported. Concerning the bone-marrow exposure we agree with the comment made. There is also no comparative in vitro metabolism study available for pethoxamide to demonstrate that metabolism of pethoxamid is comparable in mice and rat. However, within the study report and also mentioned in Annex I page 33, clinical signs including hunched posture and piloerection were reported. These clinical signs showed a dose-dependent increase in severity indicating systemic exposure at all dose levels (piloerection in the low dose group; piloerection and hunched posture in the mid-dose group; piloerection, hunched posture, ptosis, and lethargy in the highest dose group). At the highest dose of 1280 mg/kg also mortalities were reported. Please refer to the table from the original report. Therefore we conclude that there is sufficient evidence to demonstrate bone-marrow exposure but based on the clinical signs and not based on toxicokinetic data derived from rats.

RAC's response

In the in vitro chromosome aberration test in human lymphocytes (Anonymous, 1994) done according to OECD TG 473, duplicate tests were carried out. In the first test the concentrations for metaphase analysis were 2.0, 7.8 and 15.6 µg/mL without S9 mix, and 3.9, 15.6, and 31.3 µg/mL with S9 mix. In the absence of S9 mix, a statistically significant increase in chromosomal aberrations occurred at the highest dose level, 15.6 µg/mL. In the second test, without S9-mix the concentrations used for metaphase analysis were 3.75, 20, and 37.5 µg/mL, with S9-mix the concentrations were 7.5, 45 and 80 µg/mL. In the second test in the absence of S9 mix, statistically significant increases in chromosomal aberrations occurred at the intermediate and high dose levels. The percentage of cells with aberrations (excluding gaps) at 0, 3.75, 20, and 37.5 µg/mL was 0.25, 1.5, 5.5*** and 15.5***, respectively (*** = $p<0.001$). In the presence of S9 mix, a statistically significant increase in chromosomal aberrations occurred at all dose levels analysed. The percentage of cells with aberrations (excluding gaps) at 0, 7.5, 45 and 80 µg/mL was 2.5, 6.0*, 14.0*** and 41.5***, respectively (*** = $p<0.001$). The second experiment showed distinct dose-response, the assay can be deemed positive. Concerning the micronucleus test, RAC agrees with the DS that clinical signs showed a dose-dependent increase in severity (including mortalities at the highest dose), indicating systemic exposure.

Date	Country	Organisation	Type of Organisation	Comment number
09.06.2022	United States of America	FMC	Company-Manufacturer	7
Comment received				
Based on results of a battery of in vitro and in vivo genotoxicity studies, it can be concluded that pethoxamid is unlikely to be genotoxic and thus does not meet the classification criteria for germ cell mutagenicity. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Pethoxamid, FMC comments on CLH dossier, 9 June 2022.pdf				
Dossier Submitter's Response				
Noted. Thank you.				
RAC's response				
RAC has taken note of your comments.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
03.06.2022	Germany		MemberState	8
Comment received				
Developmental toxicity With respect to the rabbit study from Anonymous (2014b) a fetal NOEL of 50 mg/kg is mentioned in the table on p. 51 and the text on p. 53 of the CLH-report. In contrast, in the annex I (p. 116) the dose of 12.5 mg/kg is mentioned once as NOEL and in the next section as NOAEL. This difference with regard to the NO(A)EL might be explained.				
Dossier Submitter's Response				
Thank you for the comment. The maternal NOAEL for pethoxamide is 50mg/kg bw/day. The developmental NOAEL was set at 12.5 mg/kg bw/day.				
RAC's response				
RAC has taken note of your comment.				

Date	Country	Organisation	Type of Organisation	Comment number
09.06.2022	United States of America	FMC	Company-Manufacturer	9
Comment received				
The reproductive toxicity of pethoxamid was investigated in a rat two-generation reproduction study and in developmental toxicity studies in rats and rabbits. FMC agrees that pethoxamid does not meet the classification criteria for reproductive or developmental toxicity, since pethoxamid did not cause adverse effects on sexual function and fertility or produce evidence of developmental toxicity. FMC also agrees that pethoxamid does not meet the classification criteria for adverse effects on or via lactation. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Pethoxamid, FMC comments on CLH dossier, 9 June 2022.pdf				
Dossier Submitter's Response				
Noted. Thank you.				

RAC's response
RAC has taken note of your comment.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
10.06.2022	Switzerland	Federal Food Safety and Veterinary Office	National Authority	10

Comment received
CLH report, section 10.2.1, page 17 and annex, section 3.2.1, page 16: The latest version of the OECD guideline #402, which was adopted on 9 October 2017, states that solid test items should be moistened sufficiently, preferably with water or, where necessary, a suitable vehicle to ensure good contact with the skin. In contrast, it is stated on page 16 of the annex to the CLH report that the test material was administered as supplied to the rats at 2000 mg/kg bw to the shaved skin of each animal (application volume 1.8 mL/kg). The test material was pethoxamid, purity 95%. As pethoxamid is solid at room temperature, its direct application as supplied constitutes a deviation from the above-mentioned guideline. It seems unclear whether good contact between the test item and the skin can be ensured under such circumstances and whether robust conclusions with regard to dermal toxicity can be drawn. Please also note that the application volume – if it refers to solid Pethoxamid without vehicle –would be equivalent to ~2.14 g/kg considering the relative density of pethoxamid.
Dossier Submitter's Response
Thank you for the comment. We agree. The appearance of the test substance was stated in the original report to be: "Oily solid". Also, there is a hand-written note indicating that the substance may have been moistured with water and within the study report it is noted that the test substance was administered at 1.8ml/kg. Nevertheless, this is not certain. In addition, if the solid is oily, than solubility in water is questionable. Although as a oily solid pethoxamid would have been absorbed without moisturized, the little information given in the study report is insufficient. Therefore, this should be mentioned as deviation.
RAC's response
The CLH report states that the "substance was administered as supplied", but did not mention the physical state of the substance. The DS clarified from the archived raw data that in the acute dermal toxicity study the substance was placed in a water bath at 50°C to melt prior to dosing, and thus was administered in liquid form.

Date	Country	Organisation	Type of Organisation	Comment number
09.06.2022	United States of America	FMC	Company-Manufacturer	11

Comment received
Based on the available data, the lowest acute oral LD50 (ATE) was 983 mg/kg bw for male rats exposed to pethoxamid. Thus, FMC agrees with the proposal that pethoxamid be classified for acute oral toxicity (Acute Tox Category 4, H302). FMC agrees no classification is warranted for acute dermal and inhalation toxicity. ECHA note – An attachment was submitted with the comment above. Refer to public

attachment Pethoxamid, FMC comments on CLH dossier, 9 June 2022.pdf
Dossier Submitter's Response
Noted. Thank you.
RAC's response
RAC has taken note of your comments.

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
10.06.2022	Switzerland	Federal Food Safety and Veterinary Office	National Authority	12
Comment received				
CLH report, section 10.4.1, page 17 and Annex, section 3.4.1, page 18: The latest version of the OECD guideline #404, which was adopted on 28 July 2015, states that when testing solids the test chemical should be moistened with the smallest amount of water (or, where necessary, of another suitable vehicle) sufficient to ensure good skin contact. In contrast, it is stated on page 18 of the annex to the CLH report that the rabbits received each 0.5 mL of the test substance, which was administered as supplied to the shaved skin of each animal. The test material was pethoxamid, purity 95%. As pethoxamid is solid at room temperature, its direct application as supplied constitutes a deviation from the above-mentioned guideline. It seems unclear whether good contact between the test item and the skin can be ensured under such circumstances and whether robust conclusions with regard to potential dermal irritation/corrosion can be drawn. Also, please note that the application volume indicated in the report and the annex – if it refers to solid Pethoxamid without vehicle –is equivalent to ~0.6 g considering the relative density of pethoxamid. This would constitute another deviation from the above-mentioned guideline, which states that a dose of […] 0.5 g of solid or paste is to be applied to the test site.				
Dossier Submitter's Response				
Thank you for the comment. Unfortunately, there is no further information on the processing of the test substance. The appearance of the test substance was stated in the original report to be: "Oily solid". Six rabbits were each administered a single dermal dose of 0.5 ml of the test substance and observed for a maximum of five day". This would indicate, that the test substance may have been moistured. However, this cannot be stated with certainty. Since the performing lab was the same as for the acute dermal toxicity testing, please refer to our comment number ten.				
RAC's response				
The CLH report did not mention the physical state of the substance. The DS clarified from the archived raw data that in the skin corrosion/irritation study the substance was placed in a water bath at 50°C prior to dosing, and thus was administered in liquid form.				

Date	Country	Organisation	Type of Organisation	Comment number
09.06.2022	United States of America	FMC	Company-Manufacturer	13
Comment received				
FMC concurs that pethoxamid is not a skin irritant and that classification is not required. ECHA note – An attachment was submitted with the comment above. Refer to public				

attachment Pethoxamid, FMC comments on CLH dossier, 9 June 2022.pdf
Dossier Submitter's Response
Noted. Thank you.
RAC's response
RAC has taken note of your comment.

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
09.06.2022	United States of America	FMC	Company-Manufacturer	14
Comment received				
FMC concurs that pethoxamid is not an eye irritant and that classification is not required. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Pethoxamid, FMC comments on CLH dossier, 9 June 2022.pdf				
Dossier Submitter's Response				
Noted. Thank you.				
RAC's response				
RAC has taken note of your comment.				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
09.06.2022	United States of America	FMC	Company-Manufacturer	15
Comment received				
Based on the available data from the guinea pig maximization test (GPMT), an intradermal induction at a concentration of 0.5% (v/v) in Alembicol D, followed by a topical challenge at 25 & 12.5% (v/v) in Alembicol D, resulted in 95% of animals producing a positive response to pethoxamid. An intradermal induction concentration (v/v) of >0.1% - ≤1.0% and a challenge sensitization response of ≥60% is considered strong potency and results in a predicted subcategorization of '1A' for skin sensitization. Thus, FMC agrees with the proposal that pethoxamid be classified for skin sensitization (Skin Sensitizer Category 1A, H317). ECHA note – An attachment was submitted with the comment above. Refer to public attachment Pethoxamid, FMC comments on CLH dossier, 9 June 2022.pdf				
Dossier Submitter's Response				
Noted. Thank you.				
RAC's response				
RAC has taken note of your comments.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
09.06.2022	United States of America	FMC	Company-Manufacturer	16
Comment received				
FMC agrees that pethoxamid does not meet the classification criteria for Specific Target Organ Toxicity – Single Exposure. No single organ effect was observed in males and females exposed to a single oral (gavage) dose of pethoxamid. Furthermore, functional observation battery results indicate that pethoxamid does not cause acute neurotoxicity. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Pethoxamid, FMC comments on CLH dossier, 9 June 2022.pdf				
Dossier Submitter's Response				
Noted. Thank you.				
RAC's response				
RAC has taken note of your comments.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
10.06.2022	Switzerland	Federal Food Safety and Veterinary Office	National Authority	17
Comment received				
CLH report, section 10.12, page 71 and annex, section 3.12.1.10, page 144: The latest version of the OECD guideline #410, which was adopted on 12 May 1981, states that when testing solids, which may be pulverised if appropriate, the test substance should be moistened sufficiently with water or, where necessary, a suitable vehicle to ensure good contact with the skin. In contrast, it is stated on page 144 of the annex to the CLH report that the test material was applied neat and was covered with a semi-occlusive wrap. The test material was pethoxamid technical, purity 95.8 %. As pethoxamid is solid at room temperature, its direct application as supplied constitutes a deviation from the above-mentioned guideline. It seems unclear whether good contact between the test item and the skin can be ensured under such circumstances and whether robust conclusions with regard to dermal toxicity can be drawn.				
Dossier Submitter's Response				
Thank you for the comment. In the original study report the test material was stated as "Brown viscous liquid".				
RAC's response				
RAC has taken note of your comments.				

Date	Country	Organisation	Type of Organisation	Comment number
09.06.2022	United States of America	FMC	Company-Manufacturer	18
Comment received				
FMC agrees that pethoxamid does not meet the classification criteria for Specific Target Organ Toxicity – Repeat Exposure. Findings in rat, mouse, & dog studies reflected general				

non-specific effects, adaptive changes, or of insufficient magnitude or severity to be indicative of STOT-RE and thus no classification is appropriate. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Pethoxamid, FMC comments on CLH dossier, 9 June 2022.pdf
Dossier Submitter's Response
Noted. Thank you.
RAC's response
RAC has taken note of your comments.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
26.05.2022	United Kingdom	Health and Safety Executive	National Authority	19
Comment received				
Pethoxamid (EC: -; CAS: 106700-29-2) Although pethoxamid does not meet the CLP criteria for rapid degradation, it does undergo significant primary degradation (e.g. in a water-sediment study DT50s <16 days). Metabolites [degradants] are mentioned in the CLH report although no information is presented on their ecotoxicity. Data (e.g. toxicity to algae and Lemna) on the ecotoxicity of some metabolites (e.g. MET-6 and others) are included in the RAR. Please can you consider if these data impact the proposed hazard classification and associated M-factors.				
Dossier Submitter's Response				
Data on the toxicity of the metabolites for algae and aquatic plants are available. However, since pethoxamide clearly does not meet the CLP-criteria of "rapidly degradable" due to environmental fate and behavior, thus the toxicity of metabolites have no impact on the hazard classification and M-factor.				
RAC's response				
Based on data for primary degradation from the surface water simulation study, the substance undergoes rapid primary degradation. However, as no adequate data are available for all hydrolysis products, it cannot be excluded that the criteria for classification as hazardous to the aquatic environment are not met for these hydrolysis products. Furthermore, the data for the metabolite MET-6 (7-d EC50= 0.778 mg/L) indicates that it is classifiable. Therefore, the substance cannot be regarded as rapidly degradable for classification via primary degradation.				

Date	Country	Organisation	Type of Organisation	Comment number
10.06.2022	France		MemberState	20
Comment received				
FR agrees with the conclusion on classification and labelling for environmental hazards, i.e. Pethoxamid is classified as H400: "Very toxic to aquatic life" and H410: "Very toxic to aquatic life with long lasting effects" with the pictogram GSH09 and signal word. FR also agrees with acute and chronic proposed M-factors of 100 and 10 respectively.				
Dossier Submitter's Response				
Noted.				
RAC's response				
RAC has taken note of your comment.				

Date	Country	Organisation	Type of Organisation	Comment number
03.06.2022	Germany		MemberState	21
Comment received				
We thank the reporting member state for the assessment. The results given in Table 59 of chapter 11.1 are very clear in regard to the assessment of degradability in aquatic environment as not rapidly degradable. However, we kindly ask the dossier submitter to add a short summary of the results of the OECD 308 study in the table (e.g. showing the low measured mineralisation). Currently the results of this study are only described as "relevant for classification regarding degradability in aquatic environment", without further information on the outcome. We agree that in the current case the classification of long-term aquatic hazard should preferably be based on the ErC10. Therefore, we support the proposed classification as Aquatic Chronic 1 with an M-factor of 10, as well as the classification as Aquatic Acute 1 with an M-factor of 100.				
Dossier Submitter's Response				
BCF of Pethoxamid (steady-state and total wet weight/normalised to 6% lipid content) is 33 and the level and nature of residues in organisms after the 56 day depuration phase is > 90 %.				
RAC's response				
RAC has taken note of your comment.				

Date	Country	Organisation	Type of Organisation	Comment number
09.06.2022	United States of America	FMC	Company-Manufacturer	22
Comment received				
FMC agrees that pethoxamid meets the classification criteria for both acute and chronic aquatic ecotoxicity (Aquatic Acute 1, M-factor = 100 [H400] and Aquatic Chronic 1, M-factor = 10 [H410]). ECHA note – An attachment was submitted with the comment above. Refer to public attachment Pethoxamid, FMC comments on CLH dossier, 9 June 2022.pdf				
Dossier Submitter's Response				
Noted.				
RAC's response				
RAC has taken note of your comment.				

OTHER HAZARDS AND ENDPOINTS – Physical Hazards

Date	Country	Organisation	Type of Organisation	Comment number
10.06.2022	France		MemberState	23
Comment received				
FR: Data on explosive, flammable, self heating and oxidizing properties, are provided in the RAR (May 2017), the available results are presented in table 8 page 11 of CLH report. Nevertheless, it is indicated in the table 7 page 9 "data lacking". Please update the CLH report by including conclusions of these tests in table 7 page 9.				
Dossier Submitter's Response				
Please continue reading under Point 8, page 13. According to the comments we have received by ECHA, the used methods were not acceptable. E.g: 8.1. Explosives: ECC A.14 is not comparable to the test methods in Part I of the				

UNRTDG. Therefore data lacking. The same is true for the other physical chemical properties you have mentioned.
RAC's response
RAC has taken note of your comment.

Date	Country	Organisation	Type of Organisation	Comment number
09.06.2022	United States of America	FMC	Company-Manufacturer	24
Comment received				
FMC agrees based on the physical and chemical properties of pethoxamid that classification for physiochemical properties and physical hazards it not required. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Pethoxamid, FMC comments on CLH dossier, 9 June 2022.pdf				
Dossier Submitter's Response				
Noted.				
RAC's response				
RAC has taken note of your comment.				

PUBLIC ATTACHMENTS
1. Pethoxamid, FMC comments on CLH dossier, 9 June 2022.pdf [Please refer to comment No. 2, 5, 7, 9, 11, 13, 14, 15, 16, 18, 22, 24]