Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Opinion

proposing harmonised classification and labelling at EU level of

2-methoxyethyl acrylate

EC Number: 221-499-3
CAS Number: 3121-61-7

CLH-O-0000001412-86-202/F

Adopted

9 March 2018

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-methoxyethyl acrylate
EC number: 221-499-3
CAS number: 3121-61-7
Dossier submitter: France

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2017	United Kingdom	Envigo	Industry or trade association	1
Comment received				
Comment on the interpretation of the Comet assay mutagenicity data presented in Pages 23/24 of CLH report for 2-methoxyethyl acrylate (EC 221-499-3, CAS 3121-61-7), Version:3 February 2017, submitted by ANSES.				
Dossier Submitter's Response				
Thank you for your comment. See response to comment number 4.				
RAC's response				
RAC agrees with the proposed by DS classification as Muta. Cat. 2; H341 for the endpoint germ cell mutagenicity.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
28.04.2017	Belgium		MemberState	2
Comment received				
A clastogenic potential was highlighted in two OECD TG (476 and 473), and positive and equivocal results were obtained in a comet assay in the glandular and non-glandular stomach. BECA agrees that this dataset can support a classification as Muta. 2 since there is evidence of in vivo local genotoxicity in the stomach and of in vitro mutagenicity in two tests.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for your support.				

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2017	Netherlands	RIVM/BR	National Authority	3
Comment received				
Mutagenicity We agree with the proposed classification in category 2 for the endpoint germ cell mutagenicity. The in vitro mutagenicity tests show a positive effect of 2-methoxyethyl acrylate: mouse lymphoma assay (+/- metabolic activation) and chromosome aberration assay (+metabolic activation). Though negative results were obtained in liver and glandular stomach, the in vivo comet assay in rat points towards positive effects of the chemical in the non-glandular stomach (forestomach). It is considered that this positive effect, though in a tissue not present in humans, indicates that the chemical has mutagenic activity although this may be limited to the site of first contact. The combination of the positive results of the in vitro mutagenicity tests and the in vivo genotoxicity test are considered sufficient for classification in category 2.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
RAC agrees with the proposed by DS classification as Muta. Cat. 2; H341 for the endpoint germ cell mutagenicity.				

Date	Country	Organisation	Type of Organisation	Comment number
27.04.2017	United Kingdom	Envigo	Industry or trade association	4
Comment received				
The CLH report prepared by ANSES (Version 3 February 2017) uses the apparent low number of vehicle control animals stated in the historical control data for the glandular stomach to bring into question the adequacy of these data for the interpretation of the study results (CLP report pages 23/24) . We believe ANSES have misinterpreted these data; stating only 11 animals were used, when in fact these data are from 11 studies. We will modify the tables in a study amendment to clarify the historic control data. For the non-glandular stomach, ANSES states that the pathology report indicates dose-related cytotoxicity in the mid- and high dose tissues but only minimal cytotoxicity in the lowest dose, that no dose-related increase in tail intensity was observed with the mid-to high dose concentrations and a significant increase in tail intensity was observed in the low dose level (CLP report page 24) which it was clearly not as stated in the results table. ANSES use these statements to argue that the histopathological findings were only minimally concomitant with the Comet assay response, though they emphasise the lack of cytotoxic response in the low dose non-glandular stomach does not match a statistically significant increase in increased tail intensity which in fact did not occur. Envigo therefore believe that the observed Comet assay response was due to cytotoxicity and the lack of a dose-related increase in the non-glandular stomach in the upper two dose levels is most likely due to the tissue undergoing necrosis having reached the maximum response at the mid-dose level of 240 mg/kg: if this was a true genotoxic response we would have expected there to be a dose-related response for increased tail intensity. Paragraph 54 of the OECD 489 test Guideline states: Positive findings in the comet assay may not be solely due to genotoxicity; target tissue toxicity may also result in increases in DNA migration. Conversely, low or moderate cytotoxicity is often seen with known				

genotoxins showing that it is not possible to distinguish DNA migration induced by genotoxicity verses that induced by cytotoxicity in the comet assay alone. However, where increases in DNA migration are observed it is recommended that an examination of one or more indicators of cytotoxicity is performed as this can aid in interpretation of the findings. Increases in DNA migration in the clear evidence of cytotoxicity should be interpreted with caution.

Dossier Submitter's Response

Thank you for the clarification on the historical control data. The negative controls of the study were very low compared to the available historical control data showing high distribution of the data with a mean % of tail intensity equal to 2.6 ± 8.76 in 11 studies. ANSES gives more weight to the consistent low scores observed in the negative control group of the study than to the higher values observed with high dispersion in the historical control data of the laboratory. Nevertheless, it is agreed that results in the glandular stomach are equivocal as only 3 out of 6 animals had mean of median % tail intensity values above the highest negative control of the study in both the mid and high dose levels (See figure 1 below).

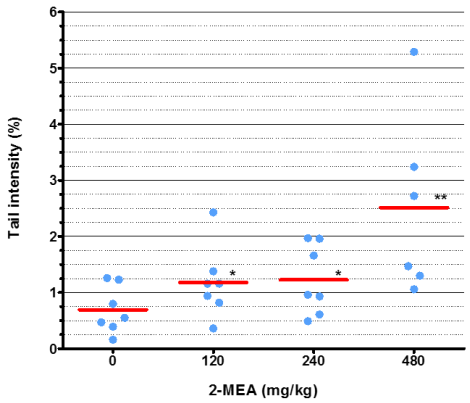

Figure 1: Comet assay data - Glandular stomach - Mean of median % tail intensity

With regards to the non-glandular stomach results, we agree that no statistically increase was observed in the low dose group (there is a mistake in page 24 of the CLH report). Please see in figure 2 below individual results in the non glandular stomach and detailed study results in annex I of the CLH. The results observed in the non glandular stomach are considered positive and reflecting true genotoxic response. Indeed, a statistically significant increase in the percentage of tail intensity was observed at the two highest doses. Although no historical control data are available for non-glandular stomach, the figure 2 below clearly shows that 6 out of 7 and 4 out of 6 animals had % of tail intensity values above the negative control of the study in the 240 and 480 mg/kg bw groups, respectively. The significant increase is thus considered related to 2-MEA genotoxic potential.

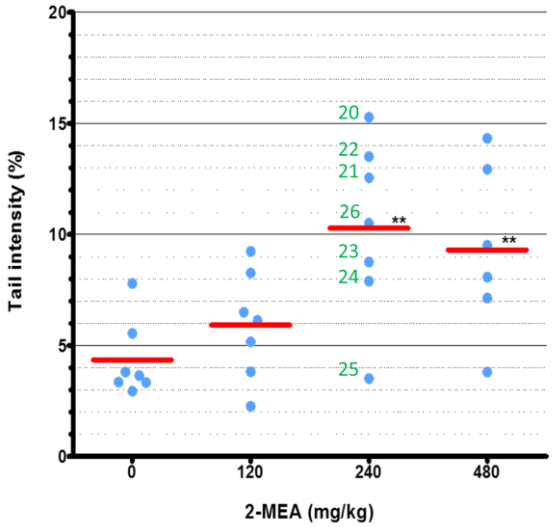

Figure 2: Comet assay data – non glandular stomach - Mean of median % tail intensity ; in green the numero of the animal in the 240 mg/kg bw dose group

The histopathological analysis of the non-glandular stomach show cytotoxic effects and more particularly at the high dose level that would suggest genotoxicity due to cytotoxicity. This argumentation is weak as the genotoxicity effects are higher at the mid dose where the cytotoxic effects are lower than in the high dose. If the genotoxicity effects were only the result of a cytotoxic response, the highest % of tail intensity in the comet assay would have been expected to be in the highest dose group, this is not the case.
We do not support the argument that tissue undergoing necrosis has reached the maximum response at the mid-dose level of 240 mg/kg bw. Indeed, as reported in table 21 of the annex I of the CLH report, marked cytotoxicity including ulceration and necrosis were only observed at 480 mg/kg and not at 240 mg/kg bw. The table below summaries the histopathological findings. Looking at the individual data in the 240 mg/kg bw dose group, it is very difficult to establish a correlation between the severity of the histopathological findings and the % of tail intensity as no histopathological effects were observed in the non-glandular stomach of animal 21 which shows a % of tail intensity above the group mean.

Table: summary of individual histopathological forestomach findings

Group	Animal No.	Findings
Control	1 to 7	No abnormalities detected
120 mg/kg	27, 28, 29, 31	No abnormalities detected
	30, 32, 33	Minimal vacuolisation of the limiting ridge
240 mg/kg	21, 25	No abnormalities detected
	23, 24	Minimal vacuolisation of the limiting ridge
	26	Minimal vacuolisation of the limiting ridge + slight epithelial hyperplasia
	20	Minimal vacuolisation of the limiting ridge + slight inflammation of submucosa + minimal myofiber degeneration
	22	Minimal focal ulceration of the limiting ridge
480 mg/kg	13, 16	Minimal to slight myofiber degeneration+ submucosa inflammation + epithelium vacuolisation + sligh mucosal necrosis

	15, 19	Minimal to moderate myofiber degeneration, submucosa inflammation ± epithelium vacuolisation ± moderate erosion + slight ulceration
	14, 18	Minimal to slight myofiber degeneration, inflammation, ± epithelium vacuolisation + slight ulceration of the limiting ridge and marked ulceration of the epithelium

In conclusion, based on the positive response observed with 2-MEA *in vitro* and the positive *in vivo* response in the non-glandular stomach in rats, classification as Muta. 2 is considered warranted for 2-MEA.

Finally, as highlighted in your comment, OECD 489 clearly stated that "*Conversely, low or moderate cytotoxicity is often seen with known genotoxins (12), showing that it is not possible to distinguish DNA migration induced by genotoxicity versus that induced by cytotoxicity in the comet assay alone*". This statement does not mean that the effects should be disregarded.

RAC's response

RAC agrees with the proposed by DS classification as Muta. Cat. 2; H341 for the endpoint germ cell mutagenicity.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
28.04.2017	Belgium		MemberState	5

Comment received

FERTILITY
In a combined repeated dose with reproduction/development screening test, BECA considers there are severe fertility effects including impairment of the spermatogenetic cycle in test, degeneration of seminiferous tubular epithelium, edema, necrosis and inflammation in the epididymis and a decrease of the fertility index. Some effects appeared at a level of 40 mg/kg bw/d where parental toxicity was not marked. In the light of these effects, there is evidence of clear fertility effect and we support the DS conclusions and proposal to classify as Repr. 1B.

DEVELOPMENT
In the same study, drastic developmental effects were seen as a decrease of the number of living pups (100, 70, 0, 0% at 0, 40, 100, 150/250 mg/kg bw/d, respectively). This is supported by a non-GLP compliant non guideline study where 100 % of intra-uterine deaths was found in mice exposed to 650 mg/kg bw/d of 2-MEA. There is no information excluding that the mechanism of toxicity is not relevant to humans. In that case, BECA agrees with the DS conclusions and with its proposal to classify as Repr. 1B – H360D.
In conclusion, BECA agrees with the following classification: Repr. 1B – H3602 FD. Two metabolites (2-methoxyethanol and methoxyacetic acid) are already classified as Repr. 1B (H360FD), supporting the DS proposal.

Dossier Submitter's Response

Thank you for your support.

RAC's response

RAC agrees with the proposed by DS classification as Repr. Cat. 1B; H360FD for the endpoint.

Date	Country	Organisation	Type of Organisation	Comment number
19.04.2017	Netherlands	RIVM/BR	National Authority	6
Comment received				
Reproductive toxicity Effects on fertility and sexual function: We agree with the proposed classification in category 1B for effects on fertility and sexual function. The data of Study Report 2012b show clear effects on fertility: effects on sperm parameters (all dose-levels, dose-related manner), reduced fertility index, reduced number of corporea lutea and implantation sites (250/150 and 100 mg/kg bw/d). Also reduced organ weights (testis, epididymides) are observed (250/150 and 100 mg/kg bw/d). Although parental toxicity was also observed, the fertility effects are not a secondary consequence to this toxicity. Effects on development: We agree with the proposed classification in category 1B for developmental effects, based on the observed developmental effects in Study Report (2012b) (i.e. reduced nr of live pups at all dose levels). The observed maternal effects cannot fully explain the observed pup effects, and it is also noticed that also at the lowest dose level developmental effects were observed (in presence of limited maternal toxicity). It is considered unlikely that at 40 mg/kg bw/day the limited maternal toxicity causes the observed developmental effects. We agree that the data of Hardin et al. (1987) cannot be used for classification, as the dose level tested in this study (i.e. 650 mg/kg bw/d) results in excessive maternal toxicity (i.e. 30% mortality in dams). The likely formation of the metabolites 2-methoxyethanol (2-ME) and methoxyacetic acid (2-MA) which also induce effects on fertility and development should be included in the comparison with the CLP criteria at least as supportive evidence				
Dossier Submitter's Response				
Thank you for your support. We agree that the likely formation of the metabolite 2-ME and 2-MA can be used as supporing evidence in the comparison with the CLP criteria.				
RAC's response				
RAC agrees with the proposed by DS classification as Repr. Cat. 1B; H360FD for the endpoint.				

RESPIRATORY SENSITISATION

Date	Country	Organisation	Type of Organisation	Comment number
28.04.2017	Belgium		MemberState	7
Comment received				
BECA agrees there is insufficient consistent predictive data to classify. Furthermore, no animal or human data is available. Positive results in animal tests fulfils the guidance criteria to classify as Skin Sens. 1. Here, subcategory is not possible. We agree to classify as proposed by the DS, and it is largely supported by already demonstrated sensitizing potential of acrylates.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
RAC agrees not to classify this substance for respiratory sensitisation, as proposed by the DS.				

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
28.04.2017	Belgium		MemberState	8
Comment received				
ORAL ROUTE Two studies equivalent to OECD TG 401 (both rel. 2) are available. In the first, SD 5 rats/sex/dose were exposed to 2-MEA. 0, 2, 2, 4 males and 0, 2, 3, 4 females died after being exposed to 252.5, 353.5, 505.0, 555.5 or 606.0 mg/kg bw, respectively. The LD50 was of 404 mg/kg bw. In the second one, 5 Wistar male rats were exposed to 2-MEA. 0, 4 and 5 rats were found dead after being treated with 505, 1010 or 2020 mg/kg bw, respectively. The resulting LD50 was 818 mg/kg bw. Both LD50 are consistent with the cat. 4 of the guidance criteria. We agree with the dossier submitter's proposal to classify 2-MEA as acute tox. 4 for the oral route. DERMAL ROUTE Not evaluated INHALATION One study similar to OECD TG 403 (rel. 2) is available. 6 males rats were exposed to 2-MEA by inhalation. 0, 3 and 6 animals were found dead between Day 1 and 3 after being exposed to 1.3 (1.4), 2.7 and 5.3 (5.4) mg/L. There seems to be a minor confusion in the doses between Table 18 and the short summary (10.3.1) as you can see above in brackets. Based on this data, LC50 was mentioned to be of 2.7 mg/L. This level fulfils the guidance criteria to be classified as Acut Tox. 3 and, therefore, BECA supports the DS proposal to classify as Acute Tox. 3 - H331.				
Dossier Submitter's Response				
Thank you for your support. As stated in table 18 and in the detailed study summary in annex I, the correct dose levels are 1.4, 2.7 and 5.4 mg/L and not 1.3 and 5.3 mg/L as mentioned in the short summary.				
RAC's response				
Thank you for your comment.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
28.04.2017	Belgium		MemberState	9
Comment received				
Two of the three studies presented can be taken into account for classification purpose. Both were conducted in the same lab (Rhôle-Poulenc Inc., in 1980) are equivalent to OECD TG 404, non-GLP and reliable with restriction. In the first study, rabbits presented a mean score of 3 for erythema and oedema at 24h and 3.17 for erythema and 2.5 for edema after 72h. No difference between intact and abraded skin was observed. In the second study, no sign of corrosion was observed at 4-h readings, but at 48-h, 5/6 animals presented skin corrosion. As necrosis was identified 48 hours after exposure, we agree the substance has to be classified. Cat 1A and 1B cannot be attributed as the exposure was longer than 1h.				

Category 1C is appropriate and we support the proposal: Skin Corr. 1C- H314.
Dossier Submitter's Response
Thank you for your support.
RAC's response
Thank you for your support.

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
28.04.2017	Belgium		MemberState	10
Comment received				
One study similar to OECD TG 405 can be used for classification. Mean 24-72h scores for the 6 rabbits were > 2 for conjunctivae redness, > 2 for conjunctivae oedema and > 1 for corneal opacity. In this case, this data set is consistent with guidance criteria for cat. 2. Considering the effects can be considered as severe since some scores were higher than 3, BECA can agree to classify as Eye. Dam 1 – H318. As this hazard is implicit to the Skin corr. 1C classification, labelling is not mandatory in order to avoid redundancy.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for your support.				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
28.04.2017	Belgium		MemberState	11
Comment received				
One LLNA test is available and results are positive, suggesting the sensitizing potential of 2-MEA. No human data is available.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Thank you for your comment.				

OTHER HAZARDS AND ENDPOINTS – Physical Hazards

Date	Country	Organisation	Type of Organisation	Comment number
28.04.2017	Belgium		MemberState	12
Comment received				
We agree that 2-methoxyethyl acrylate is a cat. 3 flammable liquid according to the flash point of 59 °C.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for your support.				