**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-(2-methoxyethoxy)ethanol; diethylene glycol monomethyl ether

**EC Number: 203-906-6**
**CAS Number: 111-77-3**

CLH-O-0000006857-59-01/F

**Adopted**

**8 October 2020**

**ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2-(2-METHOXYETHOXY)ETHANOL; DIETHYLENE GLYCOL MONOMETHYL ETHER**

**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.

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**Substance name: 2-(2-methoxyethoxy)ethanol; diethylene glycol monomethyl ether**
**EC number: 203-906-6**
**CAS number: 111-77-3**
**Dossier submitter: Netherlands**

**GENERAL COMMENTS**

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 28.06.2019 | Germany | | MemberState | 1 |
| Comment received | | | | |
| The proposed classification of DEGME as Repr. 1B (H360D) is based on developmental effects which occur mainly at doses > 1000 mg/kg bw/day. However the corresponding dose-response-relationships start at doses < 1000 mg/kg bw/day. It seems plausible that the effects seen after exposure to DEGME are caused by the metabolite 2-methoxyacetic acid (MAA), a known reproductive toxicant which is formed in limited amounts in rat. The classification of DEGME as Repr. 1B (H360D) is justified. | | | | |
| Dossier Submitter's Response | | | | |
| Thank you for your support. | | | | |
| RAC's response | | | | |
| Noted. | | | | |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 03.07.2019 | United Kingdom | Glycol Ethers REACH Consortium | Industry or trade association | 2 |
| Comment received | | | | |
| Table 3 (p2) - impurities. EGME (there is a typo in the name) is shown as being present as an impurity in the range as 0-0.4% with a potential impact on the classification. We believe that this is incorrect. In the C&L inventory, nobody has submitted a notification indicating an impurity as impacting the classification (which would add a category 1B classification in the case of EGME.) Also, EGME >=0.3% is not supported by the Substance Identity Profile in the REACH joint registration. We believe therefore it should be shown as impurity of EGME <0.3% with no impact on the classification.<br>Section 4 (p5). Justification that action is needed at a community level: Of the list of | | | | |

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| notable new information, the cited study by Groeseneken et al (1998) is on the substance 2-ethoxyethanol and metabolism to ethoxyacetic acid (EAA) and is therefore not relevant to this dossier. We propose the reference is deleted. It is also referenced in on p2, table 5 and p7 entry 5 where the references should also be deleted. We also note that there are no new studies available on the reproductive and developmental toxicity of DEGME that were not already considered when the substance classification was reviewed at EU level in 1997.<br>Section 5 (p5). Identified uses: Please note that the use in aviation fuels is for the military market (JP-8 fuels) where DEGME is used historically as a replacement for 2-methoxyethanol (EGME) due to the toxicity of the latter.<br><br>Groeseneken D, Veulemans H, Masschelein R, Van Vlem, E (1988) Comparative urinary excretion of ethoxyacetic acid in man and rat after single low doses of ethylene glycol monoethyl ether. Toxicology Letters, 41(1), 57-68.<br><br>ECHA note – An attachment was submitted with the comment above. Refer to public attachment DEGME CLH report 2019 - Response from GE REACH consortium 030719.pdf |
| Dossier Submitter's Response |
| Thank you for your response. The information on impurities was copied from the RAR (1999) as mentioned in the CLH report. It is noted that recent formulations may not contain these impurities. For this reason, we have also not included the potential presence of EGME in our justification for proposing Repr. 1B.<br>We included the references to the studies by Groeseneken et al., because they contain information on the half-life of MAA, which is also relevant for DEGME. Furthermore, these studies were not extensively discussed as part of the previous classification proposal. The studies mentioned include more information on the reproductive toxicity of MAA, which is also relevant for DEGME, and the toxicokinetics of DEGME and MAA. |
| RAC's response |
| Noted. |

**TOXICITY TO REPRODUCTION**

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 01.07.2019 | France | | MemberState | 3 |
| Comment received | | | | |
| Anses agrees with the classification proposals made by RIVM for DEGME, in particular: the malformations observed in several species (rodents, rabbits) also at low doses in the absence of maternal toxicity, and the formation of MAA as a metabolite in animals and also in humans with an higher half-life strongly support the classification as a developmental toxicant in Category 1B. | | | | |
| Dossier Submitter's Response | | | | |
| Thank you for your support. | | | | |
| RAC's response | | | | |
| Noted. | | | | |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 28.06.2019 | Germany | | MemberState | 4 |

Comment received

Developmental effects
A longer half-life of MAA in humans compared to rats is brought forward by the dossier submitter to suggest that effects may occur at lower doses in humans. With respect to species differences in toxicokinetics it is stated in chapter 3.7.2.5.7 of the CLP Regulation (p. 156): " ...due to species differences in toxicokinetics, establishing a specific limit dose may not be adequate for situations where humans are more sensitive than the animal model." However, a longer half-life in humans compared to rats can be assumed in general because of allometric scaling. Large datasets have been evaluated in this respect by e. g. Bachmann et al. (1996) and Sarver et al. (1997). As an example, an average 4-fold longer half-life in humans compared to rats is derived by Caldwell (2004). Comparing the given half-lives of MAA, which were based on urinary excretion, an about 3.5-fold longer half-life in humans can be calculated. Considering in addition half-life values of MAA, which were based on plasma levels in rats, a 3.5 to 6.0-times longer half-life in humans results. Thus, the longer half-life of MAA in humans largely reflects the general principle of allometric scaling and is not an exceptional property of MAA. It appears that in previous RAC opinions this principle higher sensitivity of humans was not mentioned as an argument in context of limit dose considerations. Thus, it should be clarified whether the higher sensitivity of humans, which can be derived by allometric scaling, should be considered. This would be relevant for the assessment of DEGME, but also in general.
Sarver et al. (1997) Estimating Xenobiotic Half-Lives in Humans from Rat Data: Influence of log P. Environmental Health Perspectives, Volume 105, Number 11, pp 1204-1209
Bachmann et al. (1996) Scaling Basic Toxicokinetic Parameters from Rat to Man. Environmental Health Perspectives, Volume 104, Number 4, pp 400-407
Caldwell et al. (2004) Allometric scaling of pharmacokinetic parameters in drug discovery: Can human CL, Vss and t1/2 be predicted from in-vivo rat data? European Journal of Drug Metabolism and Pharmacokinetics Volume 29, Issue 2, pp 133–143

Adverse effects on sexual function and fertility
p13: testicular atrophy and sperm abnormalities as reported by Krasavage and Vlaovic (1982) might be added to table 11.

Dossier Submitter's Response

Thank you for your response. Indeed, the measured longer half-life seems to correspond with the general expected increase of the half-life in humans compared to rats. However, a measurement is considered more reliable than general allometric scaling as other (unexpected) species differences may be of influence as well. Nevertheless, a half-life of about 77h in human may lead to accumulation of MAA in human following repeated exposure.
Unfortunately we cannot edit the CLH proposal anymore. As mentioned in the text, testicular atrophy (Krasavage and Vlaovic, 1982) was observed at the highest dose accompanied with clear signs of systemic toxicity.

RAC's response

Noted.

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 05.07.2019 | Belgium | | MemberState | 5 |
| Comment received | | | | |
| BE CA would thank the NL CA for this proposal to modify the classification of diethylene glycol monomethyl ether (DEGME). BE CA supports the classification of DEGME as a reprotoxicant for the development warranting a Repr. 1B, but BE CA would like to encourage the dossier submitter to review its opinion on fertility and to conclude with more precaution on this endpoint.<br><br>Reproductive toxicology<br>Fertility<br>BE CA is concerned by the presence of the impurity 2-methoxyethanol (2-MEA) in DEGME. Indeed, this chemical is already classified as Repr. 1B for fertility and development. As the dossier submitter specified in the CLH dossier, "in a mixture, a substance has to be classified when the concentration of the reproductive toxicant is above 0.3 %". BECA agrees with NL CA that 2-MEA concentration in DEGME ranges from 0 to 0.4 %, and that it is not clear when and how 2-MEA can be found at a concentration of 0.3 % and above. However, BE CA is of the opinion that 2-MEA presence in DEGME should be taken into account for classification, considering a concentration at 0.4 % (thus legally warranting a classification) cannot be excluded. Furthermore, BECA would like to stress that classify or not classify a substance depending of the concentration of a classified impurity in the batch seems to be purely idealistic.<br><br>Development<br>BE CA supports the assessment and the conclusions proposed by the dossier submitter on the developmental effects of DEGME, and agrees that the substance requires a classification as Repr. 1B for development. | | | | |
| Dossier Submitter's Response | | | | |
| Thank you for your support. We recognise it may be idealistic to have a composition classified based on variable concentrations of an impurity at concentrations around the concentration limit for classification. The presence of the impurity is based on the RAR (1999). It is unclear whether this impurity can still be present or not. According to the DEGME consortium, it is not present (anymore) in quantities warranting classification. Therefore, it is not possible to propose classification based on this impurity. | | | | |
| RAC's response | | | | |
| Noted. | | | | |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 26.06.2019 | Finland | | MemberState | 6 |
| Comment received | | | | |
| Developmental effects of 2-(2-methoxyethoxy)ethanol (DEGME) have been studied in a number of non-guideline oral rat studies. In one study (similar to OECD TG 414 but with deviations), the number of live births per litter and foetal weight were both significantly reduced compared to control group. Clear, seemingly dose-dependent skeletal and visceral malformations were also observed in the absence of maternal toxicity. In another rat study (similar to TG 414 but with deviations), foetal body weight was significantly decreased, postnatal mortality significantly increased, and the incidence of skeletal and visceral malformations significantly increased compared to controls.<br>Regarding reproductive information on DEGME in humans, there is one case report | | | | |

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| presenting developmental effects but lacking adequate exposure data. Approximately 1% of the substance is metabolized in rats to 2-methoxyacetic acid (MAA), which is classified due to its teratogenicity (Repr. Cat. 1B). According to ECETOC, MAA is likely to be responsible of the adverse reproductive outcomes of DEGME. Due to longer half-life and accumulation potential of MAA in humans, developmental effects might occur in humans at lower exposure levels than in rats. Based on the available information, FI CA supports the proposal to classify DEGME as a Repr. 1B, H360D. |
| Dossier Submitter's Response |
| Thank you for your support. |
| RAC's response |
| Noted. |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 03.07.2019 | United Kingdom | Glycol Ethers REACH Consortium | Industry or trade association | 7 |

Comment received

Please refer to the non-confidential attachment for a detailed critique of the CLH report.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment DEGME CLH report 2019 - Response from GE REACH consortium 030719.pdf

Dossier Submitter's Response

The triskelion study should indeed be "reliable without restriction".
Table 10 values were given in ranges and derived from a draft report. The proposed values by the consortium, are mostly within these ranges and can be accepted. The proposed table with differences in red:

| | 500mg/kg | 1000mg/kg | 2000mg/kg |
|---|---|---|---|
| **MEAA** | 94.5 (7.7) | 91.1 (8.5) | 87.2 (4.4) |
| **MAA** | 1.4 (0.1) | 1.1 (0.1) | 0.8 (0.1) |
| **DEG** | 2.4 (0.3) | 1.7 (0.6) | 1.6 (0.3) |
| **DEGME-glucoronide** | 1.0 (0.1) | 0.8 (0.1) | 0.7 (0.1) |
| **DEGME** | 3.4 (0.4) | 3.6 (0.7) | 4.9 (0.7) |

The information on MAA is the most important and the modified value (1.4) is the upper range value from table 10 (0.8-1.4).
Regarding the half-life of MAA, which is slower in humans compared to rats, it is true we did not consider formation of MAA in the first place. As MAA has to be formed first, the DS agrees the rate of formation might be different in humans compared to rats. However, it is not known how large this difference is, and whether the rate is higher in humans or in rats. The provided studies estimated human equipotent doses using PBPK modelling and the formation rate of MAA in humans was derived *in vitro* using primary hepatocytes metabolising EGME, EGBE and EGEE (not DEGME). The DS was unable to retrieve the original publication on the formation rate and could not evaluate important aspects of the study, such as the number of donors for the primary hepatocytes. Nevertheless, the high half-life of MAA in humans indicates bioaccumulation upon repeated exposure. In case it will be proven that the rate of formation of MAA in humans is considerably lower, the net result may be a similar sensitivity in humans compared to rats based on kinetic information. We agree such situation for DEGME may be expected based on the limited information available and therefore humans may not be much more sensitive, but it cannot be excluded either.

We agree that in principle the effects up to the limit dose are relevant for classification. However, the effects seen at dose levels above the limit dose are still relevant in an indirect way because they indicate a dose-response relationship and suggest the effects at the limit dose level may be sufficient for classification. To clarify if effects may occur at the limit dose level with an adequate study under OECD test guidelines, the DEGME consortium proposes to perform a new study and put the CLH process "on hold".
The DS has to decline this proposal for the following reasons:

- The study will reveal if DEGME is able to cause effects at limit dose levels that can be considered severe enough for classification in category 1B. However, we do not think such an animal intensive study is justified considering that DEGME is a low potency reproductive toxicant and if classified as Repr. 1B. it will get the same specific concentration limit as the general concentration limit that currently applies with Repr. 2, which is 3%. Therefore the impact will remain somewhat limited, regardless of the classification. The protection of human health will likely be sufficient with either classification.
- This classification proposal was submitted because the Dutch Health Council is of the opinion that based on the current data, DEGME should be classified as Repr. 1B. This led to a more stringent classification in The Netherlands. Therefore this proposal is submitted to harmonize the classification between The Netherlands and Europe.
- It is not possible to put the process on hold and await the results of a new study. The CLH proposal would have to be withdrawn instead and resubmitted after evaluation of the new study. The CLH process will need to be restarted after evaluation of the results and it may easily take up to 4 years before the new proposal is discussed by the RAC (the proposed study will also have to be accepted by the MSC).
- No TPE was submitted to ECHA at the start of writing this response. Furthermore, it is not guaranteed that the MSC will accept the TPE and the study may not be performed. As a result, there is a risk much time and additional resources would have to be committed to this dossier without any additional value.

To get a better idea of potential effects at the limit dose level, one of our benchmark dose (BMD) experts has analysed the data from the two developmental studies with rats by Hardin et al. (1986) and Yamano et al. (1993), using the BMD software PROAST (versions 66.40 and 66.41, https://www.rivm.nl/en/proast) in line with the EFSA guidance on BMD analysis (EFSA, 2017). The BMD confidence intervals (denoted by the 95% lower (BMDL) and 95% upper (BMDU) confidence limits) were derived for the following effects: reduction in pup weight, cardiac malformations, visceral (including cardiac) malformations, rib malformations, skeletal (including rib) malformations and viability on PND 4. Notably, this analysis included dose levels up to 2400 mg/kg bw/day as we agree that higher dose levels resulted in maternal toxicity. Up to 2400 mg/kg bw/day, maternal toxicity was small (<10% lower maternal body weights etc.) and therefore the reproductive effects at this level are considered relevant. Note that the EPA (2015) has also performed BMD analyses on various endpoints using the same studies but they did not report the BMD confidence interval, but just the BMDL. The EPA analysis was performed with interest in other endpoints for the purpose of risk assessment (point of departures), rather than classification. They did perform a similar analysis on the endpoint foetal weights, which resulted in $BMDL_{05}$ values within the same order of magnitude as calculated by our expert. Differences in BMDLs may be explained by the application of covariate analysis in the current BMD analysis.
At the start of the analyses, the eight datasets (male and female data from the dose range finder and main studies by Hardin et al. and Yamano et al.) were compared for the (continuous) endpoint of foetal body weight to determine whether the dose-responses of the eight datasets were statistically similar or different using covariate analysis (Fig. 1). Both sexes turned out to be equally sensitive. In addition, the datasets from the two main studies and the dose range finder study by Yamano et al. (1993), did not result in

statistically significant different sensitivity. Therefore, one BMD confidence interval could be derived for these three datasets, which is more precise due to the higher number of data informing the BMD confidence interval. This is also one of the reasons why the calculated $BMDL_{05}$ is slightly different from the one calculated by the EPA.

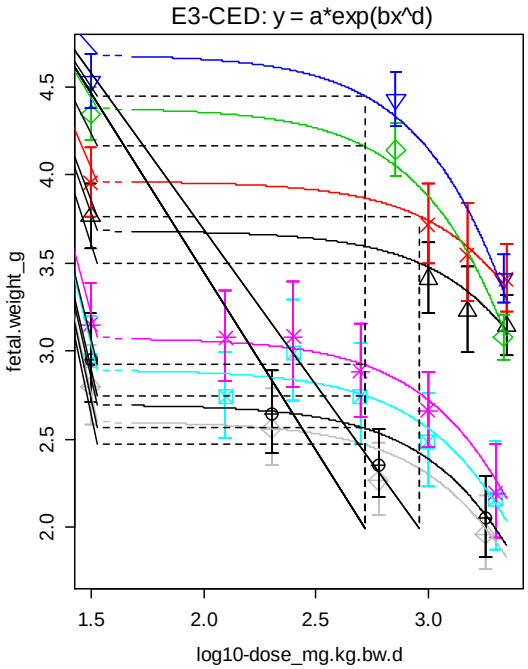


Fig 1: dose-response analysis of foetal weights. Vertical dashed lines represent two BMDs, one of both sexes in the dose finding study by Harding et al. (1986), (red X and black triangle), and one of both sexes in the two main studies and the dose range finder study by Yamano et al. (1993), (all other curves). See Table 1 for corresponding BMDLs and BMDUs.

The results have been summarized in Table 1 (summarised data tables and example dose-response figures derived from the studies and used for the BMD analyses can be found at the end of this response section).
The studies in the table were abbreviated as follows:
Har_do.fi - dose range finding study by Hardin et al. (1986)
Har_main - main study by Hardin et al. (1986)
Yam_do.fi - dose range finding study by Yamano et al. (1993)
Yam_main - main study by Yamano et al. (1993)
BMR – Bench Mark Response

| **Endpoint**<br>**BMR** | **subgroup** | **BMD conf. interval models combined** (mg/kg bw/day) @ | |
|---|---|---|---|
| | | **BMDL**** | **BMDU#** |
| Foetal Weight | | | |
| 5% decrease | Har_do.fi | 700 | 1200 |
| | Har_main & Yam_do.fi & Yam_main | 390 | 680 |
| | | | |
| 10% decrease | Har_do.fi | 1300 | 2000 |
| | Har_main & Yam_do.fi & Yam_main | 740 | 1100 |
| | | | |

| | | | |
|---|---|---|---|
| Skeletal malformations[$] | | | |
| 10% extra risk | har_do.fi | 1100 | 1600 |
| | Har_main | 600 | 980 |
| | Yam_main | 1500 | 4300 |
| | | | |
| 1% extra risk | har_do.fi | 280 | 740 |
| | Har_main | 90 | 340 |
| | Yam_main | 630 | 9800 |
| | | | |
| Wavy/fused ribs, bilateral | | | |
| 10% extra risk | har_do.fi | 1500 | 2400 |
| | har_main | 860 | 1600 |
| | | | |
| 1% extra risk | har_do.fi | 460 | 1200 |
| | har_main | 160 | 550 |
| | | | |
| Cardiovascular malformations | | | |
| 10% extra risk | har_do.fi | 1700 | 2400 |
| | Har_main & yam_main | 1100 | 1500 |
| | | | |
| 1% extra risk | har_do.fi | 700 | 1400 |
| | Har_main & yam_main | 510 | 910 |
| | | | |
| Visceral malformations* | | | |
| 10% extra risk | har_do.fi | 910 | 1500 |
| | Har_main | 910 | 1500 |
| | | | |
| 1% extra risk | har_do.fi | 190 | 850 |
| | har_main | 190 | 830 |
| | | | |
| Pup mortality on PND 4 | | | |
| 10% extra risk | yam_main | 260 | 510 |
| | | | |
| 1% extra risk | yam_main | 46 | 250 |

*incl. cardiovascular malformations
@ rounded to 2 sign. numbers
** for foetal W: lowest BMDL from expo and Hill model; for malformations based on model averaging of 8 models
# for foetal W: highest BMDU from expo and Hill model; for malformations based on model averaging of 8 models
$ The skeletal malformations were reported as such by the studies. However, we would consider most of these as malformations or variations with a low concern (ECETOC, 2002).

It is likely more than 10% reduction in foetal body weights will be achieved at dose levels below 1000 mg/kg bw/day since the $BMD_{10}$ confidence interval of the main studies is mostly below the limit dose level. Considering these studies have a shorter dosing regime, it is likely a foetal weight reduction of at least 10% will be observed at the limit dose level with a more sensitive modern OECD TG414 study. The BMD based on the dataset from the dose range finding study by Hardin et al. (1986), appeared less sensitive compared to the main studies and the dose range finder study by Yamano et al. (1993).

*Skeletal Malformations*
Total skeletal malformations were assessed separately because the studies did not investigate exactly the same malformations. Note that the level of detail in the reported malformations from the dose range finder study by Yamano et al. (1993), was insufficient for BMD analysis. Below limit dose levels, a BMD confidence interval corresponding to a 10% extra risk for skeletal malformations was calculated based on the main study by Hardin et al., (1986) but not based on the other studies, although a small part of the confidence interval calculated from the main study by Yamano et al. (1993) lies below the limit dose. However, this study reported different malformations that were only observed at the highest dose level. As a result, the upper confidence limit is very high and the sensitivity of the study is low.
At a 1% increased incidence level, both studies by Hardin et al. indicate some skeletal malformations will develop below the limit dose level. However, most of the malformations as presented in the publication are actually variations or malformations with a low level of concern. For example, the rudimentary cervical rib malformations should be considered as a low level of concern (ECETOC, 2002). Most of the other malformations were rib malformations that were reported as wavy or fused. Wavy ribs are currently associated with variations (not of high concern) but fused ribs are considered as severe/high concern (ECETOC, 2002). Unfortunately no distinction was made between these two malformations in the publication. A BMD analysis on the bilateral fused/wavy ribs was performed separately as well. The unilateral fused/wavy ribs were not included as these were lower in number and it was not clear if some of these were from the same animals that may also have had the bilateral fused/wavy ribs. From the main study by Hardin et al. (1986), it can be concluded that a risk of >1% cannot be excluded below the limit dose (of 1000 mg/kg bw/day) for the combination of fused/wavy ribs, but it is unclear how many of them are actually fused and can be considered severe. Therefore this finding may only be considered as supportive for classification in a weight of evidence approach.

*Visceral and cardiac malformations*
The visceral malformations consisted mostly of cardiac malformations. As a results, the derived BMD confidence intervals are similar. In the study by Yamano et al. (1993), the visceral malformations reported consisted only of cardiac malformations and therefore the BMD confidence interval on visceral malformations (not shown in Table 1) is the same as for cardiac malformations. As the sensitivity to develop cardiac malformations was essentially the same in both main studies, one and the same BMD confidence interval could be derived for both main studies. The $BMD_{01}$ confidence interval of the main studies on cardiac malformations indicates a >1% risk of malformations at doses below the limit dose level. As these malformations are of high concern and typical for exposure to MAA, the DS is of the opinion they are relevant for classification.

*Postnatal mortality*
Yamano et al. (1993) also investigated the postnatal viability. As mentioned in the CLH proposal, postnatal viability decreased significantly at 1800 mg/kg bw/day and non-significantly at 600 mg/kg bw/day. BMD analysis on this endpoint indicates >10% risk of postnatal mortality on PND4 at or below limit dose levels. This is considered a severe and clear adverse reproductive effect at relevant concentrations for classification.

In conclusion, the BMD analyses clarifies it is highly likely that toxicologically significant effects (>1% cardiac malformations, >10-% lower foetal pup weights, >10% pup mortality) will occur at or below the limit dose level. Therefore in the end, the DS remains of the opinion that DEGME should be classified as a reproductive toxicant in category 1B, but with low potency and the corresponding SCL of 3%.

Other remarks
The consortium requests to remove some content from the CLH report as they think it is not supportive or unreliable information. For example, the case study referred to without clear exposure information.
We repeat that no modifications can be made at this point. Furthermore, we think the uncertainty of the findings, especially regarding exposure have been sufficiently described and therefore the information should remain included in the CLH report.

The consortium further requests to include Hermsen et al. (2011), which investigated the effects of glycol ethers and their metabolites on developing embryos of zebrafish. The metabolites were toxic, while the parent compounds were not. We agree this supports the hypothesis the metabolite MAA is likely responsible for the effects, while DEGME itself may not cause developmental effects. However, this study cannot exclude whether DEGME causes developmental toxicity in another way in rats and humans.
The consortium also refers to Toraason et al. (1986), who noted similar effects caused by MAA after about half the dose as compared to EGME. As EGME has a NOAEL of 25 mg/kg bw/day, MAA should have a NOAEL of about half this level even though it has never been tested at this dose level. In addition, the consortium considers that after exposure to DEGME, approximately, 1% is metabolised to MAA at limit dose levels, yielding MAA concentrations slightly below the derived NOAEL for MAA (1% of 1000 mg/kg bw/day -> 10 mg/kg bw/day < 12.5 mg/kg bw/day). This extrapolation seems uncertain as the dose-effect levels for both chemicals in comparison to the NOAEL of both chemicals may not be linearly related. This would also presume a concentration of DEGME of 1250 mg/kg bw/day or higher may yield sufficient effects, which is not that far above the limit dose. In addition, NOAELs depend heavily on the experimental setup and on the quality of the experiment (Edler et al., 2002, Crump et al., 1984, Leisenring and Ryan, 1992, WHO 1999). The NOAEL may be influenced by e.g. group size, between subject variability, experimental error, dose spacing and dose placement. In addition the NOAEL does not correspond to a (predefined) effect size and the uncertainty in the NOAEL cannot be quantified. Furthermore, due to the pair-wise statistical comparison between control and treatment groups, information from the other dose groups remains unused when deriving a NOAEL. BMD analysis circumvents these drawbacks, and therefore, we think BMD analyses of the current data provides better information whether DEGME is likely/unlikely able to cause toxicologically relevant developmental effects at limit dose levels.

The consortium attempted to calculate an ED10 as it may be informative for a critical concentration where heart malformations may be seen. All of the calculated ED10s are above the limit dose. The analysis method and software is not provided. In our BMD analyses the $BMD_{10}$ confidence intervals calculated for cardiac malformations were also above the limit dose. Therefore we agree the ED10 for this endpoint is above the limit dose level and DEGME can be considered to have low potency for causing cardiac malformations. As mentioned before, we do consider a 1% incidence already relevant for classification. In addition, the $BMD_{10}$ for foetal weight and postnatal viability were below the limit dose. Interestingly, the $BMD_{10}$ for postnatal viability was also mostly below the threshold for a low potency reproductive toxicant (suggesting higher potency). However, as the upper confidence limit exceeds the threshold of 400 mg/kg bw/day and most other parameters have clearly lower potency, the DS agrees DEGME may be considered to have low potency for reproductive effects in line with the CLP criteria.

In conclusion, the DS remains of the opinion DEGME should be classified as a reproductive toxicant in category 1B for effects on development (H360D) because:

- Increased cardiac malformations and postnatal mortality starting at concentrations below the limit dose and reaching statistical significance at concentrations above the limit-dose in the rat in the absence of maternal toxicity. BMD analyses indicates DEGME is expected to cause at or below the limit dose:
    - an increase of 10% skeletal variations/malformations (with an unclear fraction of high concern malformations),
    - a reduction in foetal body weight of at least 10%,
    - an increase of >1% cardiac malformations
    - An increase of >10% postnatal mortality at PND4 (already present at PND2).
- Formation of 2-methoxyacetic acid in potentially teratogenic amounts (1% is 10 mg/kg bw/day at the limit dose while it causes malformations from 39 mg/kg bw/day in rats, but lower concentrations have not been tested).
- The half-life of MAA is slower in humans compared to rats and it may accumulate after repeated exposure. It is acknowledged the formation rate of MAA is expected to be slower as well, but the relevant source of this information could not be evaluated and it therefore remains unclear if MAA is indeed formed more slowly in humans compared to rats resulting in equipotency in both species.

Overall these observations indicate clear developmental toxicity that are likely occurring at dose levels at or below the limit dose, warranting classification as Repr. 1B, H360D.

<u>Background information: Data used for BMD analyses:</u>
Tables with data derived from the studies and used for the BMD analyses:
Note that Yamano et al. (1993), reported SEMs of foetal weights, which were transformed into SDs.

Foetal weight:

| Dose (mg/kg bw/day) | ref | study | sex | study_sex | Mean Foetal weight (g) | Foetal weight SD | No. of pups (n) | Foetal weight SEM |
|---|---|---|---|---|---|---|---|---|
| 0 | har | har_do.fi | m | har_do.fi_m | 4 | 0,6 | 55 | NA |
| 1000 | har | har_do.fi | m | har_do.fi_m | 3.8 | 0.8 | 38 | NA |
| 1495 | har | har_do.fi | m | har_do.fi_m | 3.6 | 0.6 | 23 | NA |
| 2235 | har | har_do.fi | m | har_do.fi_m | 3.5 | 0.8 | 43 | NA |
| 0 | har | har_do.fi | f | har_do.fi_f | 3.8 | 0.5 | 55 | NA |
| 1000 | har | har_do.fi | f | har_do.fi_f | 3.5 | 0.8 | 38 | NA |
| 1495 | har | har_do.fi | f | har_do.fi_f | 3.3 | 0.7 | 23 | NA |
| 2235 | har | har_do.fi | f | har_do.fi_f | 3.2 | 0.6 | 43 | NA |
| 0 | har | har_main | m | har_main_m | 4.6 | 0.8 | 126 | NA |
| 720 | har | har_main | m | har_main_m | 4.5 | 0.8 | 113 | NA |
| 2165 | har | har_main | m | har_main_m | 3.5 | 0.8 | 86 | NA |
| 0 | har | har_main | f | har_main_f | 4.4 | 0.7 | 126 | NA |
| 720 | har | har_main | f | har_main_f | 4.2 | 0.7 | 113 | NA |
| 2165 | har | har_main | f | har_main_f | 3.2 | 0.9 | 86 | NA |
| 0 | yam | yam_dr.fi | m | yam_dr.fi_m | 3.2 | 0.56 | 49 | 0.08 |
| 125 | yam | yam_dr.fi | m | yam_dr.fi_m | 3.1 | 0.36 | 37 | 0.06 |
| 250 | yam | yam_dr.fi | m | yam_dr.fi_m | 3.1 | 0.32 | 28 | 0.06 |
| 500 | yam | yam_dr.fi | m | yam_dr.fi_m | 3.4 | 2.12 | 31 | 0.38 |
| 1000 | yam | yam_dr.fi | m | yam_dr.fi_m | 2.7 | 0.45 | 41 | 0.07 |
| 2000 | yam | yam_dr.fi | m | yam_dr.fi_m | 2.2 | 0.17 | 18 | 0.04 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 0 | yam | yam_dr.fi | f | yam_dr.fi_f | 3 | 0.56 | 39 | 0.09 |
| 125 | yam | yam_dr.fi | f | yam_dr.fi_f | 2.8 | 0.57 | 33 | 0.1 |
| 250 | yam | yam_dr.fi | f | yam_dr.fi_f | 3 | 0.22 | 29 | 0.04 |
| 500 | yam | yam_dr.fi | f | yam_dr.fi_f | 3.2 | 1.91 | 24 | 0.39 |
| 1000 | yam | yam_dr.fi | f | yam_dr.fi_f | 2.5 | 0.29 | 23 | 0.06 |
| 2000 | yam | yam_dr.fi | f | yam_dr.fi_f | 2.2 | 0.40 | 13 | 0.11 |
| 0 | yam | yam_main | m | yam_main_m | 3.3 | 1.64 | 93 | 0.17 |
| 200 | yam | yam_main | m | yam_main_m | 2.9 | 1.30 | 86 | 0.14 |
| 600 | yam | yam_main | m | yam_main_m | 2.6 | 1.21 | 101 | 0.12 |
| 1800 | yam | yam_main | m | yam_main_m | 2.1 | 0.44 | 54 | 0.06 |
| 0 | yam | yam_main | f | yam_main_f | 3.1 | 1.47 | 96 | 0.15 |
| 200 | yam | yam_main | f | yam_main_f | 2.8 | 1.23 | 90 | 0.13 |
| 600 | yam | yam_main | f | yam_main_f | 2.5 | 1.16 | 80 | 0.13 |
| 1800 | yam | yam_main | f | yam_main_f | 2 | 0.38 | 57 | 0.05 |

Malformations:

| dose (mg/kg bw/day) | ref | study | Skeletal malformations | Skeletal malformations (n) | Visceral malformations | Visceral malformations (n) | Cardiovascular malformations | Cardiovascular malformations (n) | Wavy/Fused Ribs Bilateral | Wavy/Fused Ribs Bilateral (n) | Dead PupsDay4 | Viable Pups Day 0.(n) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | har | har_do.fi | 1 | 55 | 2 | 54 | 0 | 54 | 0 | 55 | NA | NA |
| 1000 | har | har_do.fi | 2 | 38 | 6 | 38 | 1 | 38 | 2 | 38 | NA | NA |
| 1495 | har | har_do.fi | 4 | 23 | 4 | 23 | 0 | 23 | 2 | 23 | NA | NA |
| 2235 | har | har_do.fi | 13 | 42 | 15 | 44 | 7 | 44 | 6 | 42 | NA | NA |
| 0 | har | har_main | 6 | 123 | 3 | 129 | 0 | 129 | 4 | 123 | NA | NA |
| 720 | har | har_main | 15 | 111 | 4 | 115 | 1 | 115 | 6 | 111 | NA | NA |
| 2165 | har | har_main | 45 | 89 | 37 | 82 | 33 | 82 | 32 | 89 | NA | NA |
| 0 | Yam | yam_main | 0 | 91 | NA | NA | 0 | 98 | NA | NA | 8 | 100 |
| 200 | Yam | yam_main | 0 | 85 | NA | NA | 0 | 91 | NA | NA | 6 | 101 |
| 600 | Yam | yam_main | 0 | 88 | NA | NA | 1 | 93 | NA | NA | 35 | 93 |
| 1800 | Yam | yam_main | 5 | 52 | NA | NA | 18 | 59 | NA | NA | 35 | 37 |

Example analyses foetal weight (continuous data):
Basically, the approach was to fit the data with various dose-response models, and to determine if there was a significant difference in sensitivity between subgroups (e.g. sexes, studies) using covariate analysis. When subgroups showed no difference in sensitivity, one BMD confidence interval was obtained for all subgroups (Slob, 2002).

Figure 2 below shows the dose-response curves fitted with the Hill model for a reduction in foetal weight. Note that figure 1 (above) shows the fit of the exponential model to the same data.

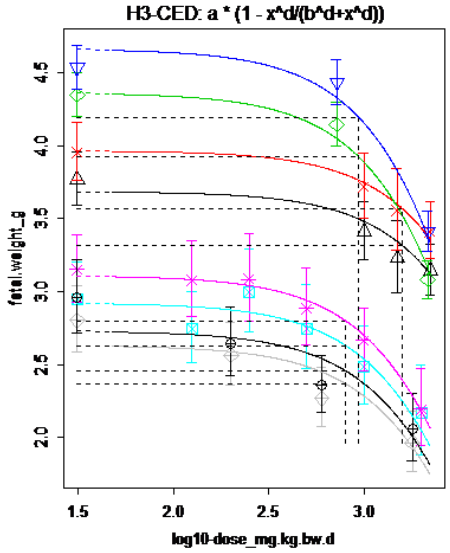


Figure 2.
The confidence intervals (BMDL – BMDU) for a 10% reduction in foetal body weight calculated using the Hill model, are:
har_do.fi 1290 - 1990
har_main_yam 744 - 1070
Lowest and highest confidence from the exponential and Hill models combined are presented in the main table (1)

Example analyses cardiac malformations (1% risk, quantal data):

When using study 2 as covariate, there was no difference between har_main and yam_main. Eight models were fitted to the data. Model averaging (EFSA, 2017) was applied to calculate the confidence intervals of the $BMD_{10}$ and $BMD_{01}$.

### Fitted Models

| model | No.par | loglik | AIC | accepted | BMDL | BMDU | BMD | sens.subgr | conv |
|---|---|---|---|---|---|---|---|---|---|
| null | 1 | -217.64 | 437.28 | | NA | NA | NA | | NA |
| full | 10 | -134.21 | 288.42 | | NA | NA | NA | | NA |
| two.stage-b | 4 | -133.15 | 274.30 | no | NA | NA | 1050 | har_yam_main | no |
| log.logist-b | 4 | -128.47 | 264.94 | yes | 1110 | 1430 | 1270 | har_yam_main | yes |
| Weibull-b | 4 | -128.62 | 265.24 | yes | 1130 | 1460 | 1300 | har_yam_main | yes |
| log.prob-b | 4 | -128.61 | 265.22 | yes | 1040 | 1350 | 1190 | har_yam_main | yes |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| gamma-b | 4 | -128.51 | 265.02 | yes | 1090 | 1390 | 1240 | har_yam_main | yes |
| logistic-a | 3 | -134.07 | 274.14 | no | NA | NA | 1560 | – | yes |
| LVM: Expon. m3-a | 4 | -128.72 | 265.44 | yes | 1100 | 1460 | 1260 | har_yam_main | yes |
| LVM: Hill m3-a | 4 | -128.64 | 265.28 | yes | 1070 | 1440 | 1230 | har_yam_main | yes |

### Weights for Model Averaging

| two.stage | log.logist | Weibull | log.prob | gamma | logistic | EXP | HILL |
|---|---|---|---|---|---|---|---|
| 0 | 0.19 | 0.16 | 0.16 | 0.18 | 0 | 0.15 | 0.16 |

### Final BMD Values

| subgroup | BMDL | BMDU |
|---|---|---|
| har_do.fi | 1690 | 2350 |
| har_yam_main | 1130 | 1460 |

Confidence intervals for the BMD are based on 200 bootstrap data sets.

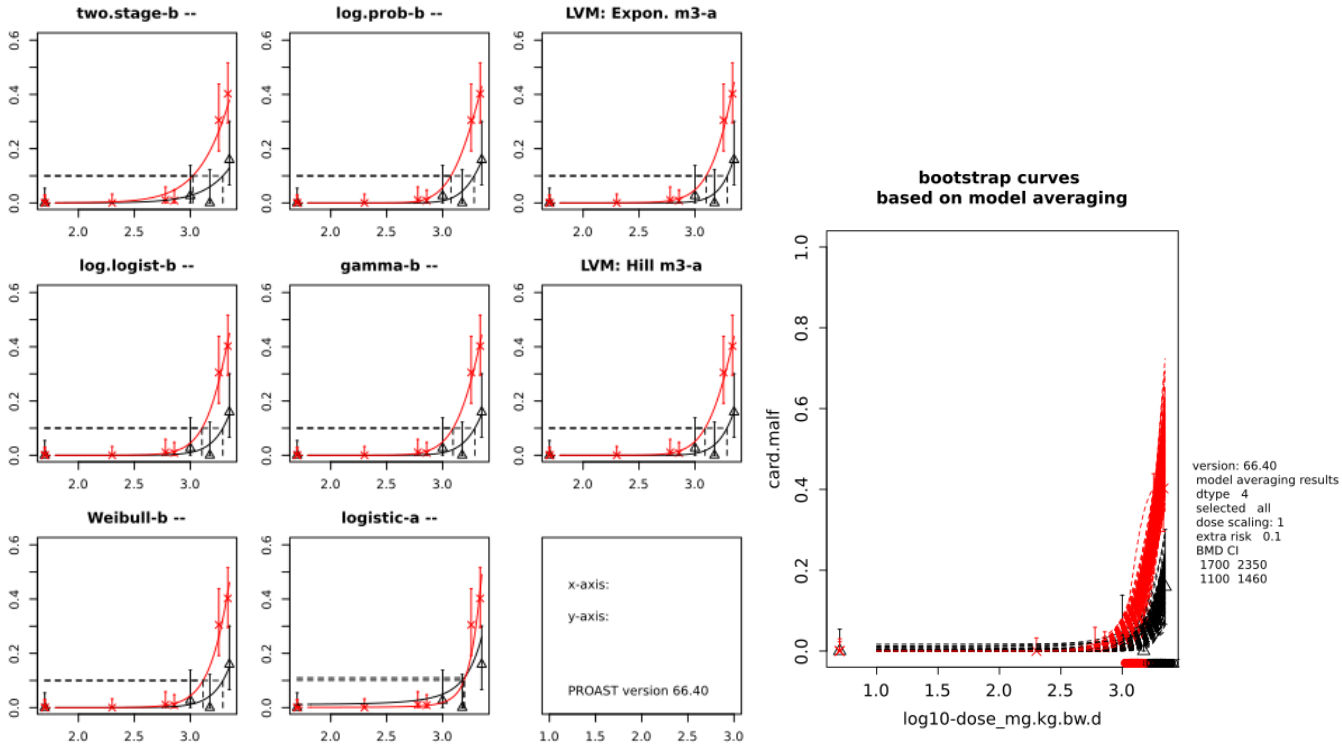


Figure 3. Dose-response model fits of quantal data (cardiac malformations) with calculations for the $BMD_{10}$ (extra risk 0.1 or 10%).

### Fitted Models

| model | No.par | loglik | AIC | accepted | BMDL | BMDU | BMD | sens.subgr | conv |
|---|---|---|---|---|---|---|---|---|---|
| null | 1 | -217.64 | 437.28 | | NA | NA | NA | | NA |
| full | 10 | -134.21 | 288.42 | | NA | NA | NA | | NA |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| two.stage-b | 4 | -133.15 | 274.30 | no | NA | NA | 325 | har_yam_main | yes |
| log.logist-b | 4 | -128.47 | 264.94 | yes | 468 | 844 | 644 | har_yam_main | yes |
| Weibull-b | 4 | -128.62 | 265.24 | yes | 452 | 827 | 633 | har_yam_main | yes |
| log.prob-b | 4 | -128.61 | 265.22 | yes | 504 | 839 | 651 | har_yam_main | yes |
| gamma-b | 4 | -128.51 | 265.02 | yes | 479 | 838 | 647 | har_yam_main | yes |
| logistic-a | 3 | -130.37 | 266.74 | yes | 540 | 946 | 730 | – | yes |
| LVM: Expon. m3-a | 4 | -128.72 | 265.44 | yes | 428 | 811 | 625 | har_yam_main | yes |
| LVM: Hill m3-a | 4 | -128.64 | 265.28 | yes | 449 | 813 | 638 | har_yam_main | yes |

### Weights for Model Averaging

| two.stage | log.logist | Weibull | log.prob | gamma | logistic | EXP | HILL |
|---|---|---|---|---|---|---|---|
| 0 | 0.17 | 0.15 | 0.15 | 0.17 | 0.07 | 0.14 | 0.15 |

### Final BMD Values

| subgroup | BMDL | BMDU |
|---|---|---|
| har_do.fi | 696 | 1420 |
| har_yam_main | 514 | 905 |

Confidence intervals for the BMD are based on 200 bootstrap data sets.

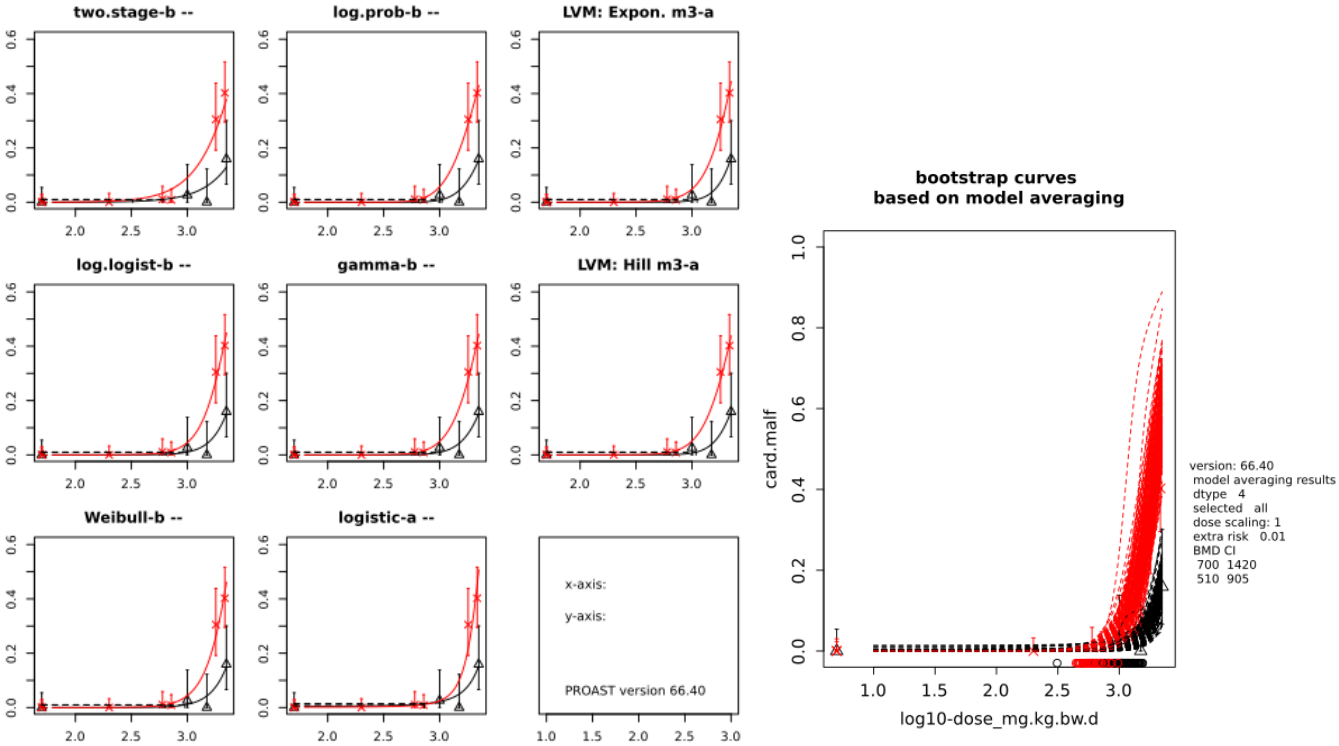

Figure 4. Dose-response model fits of quantal data (cardiac malformations) with calculations for the $BMD_{01}$ (extra risk 0.01 or 1%).

Additional references:

Crump, K.S. (1984) A new method for determining allowable daily intakes. *Fundam Appl Toxicol* **4**, 854-71.

ECETOC (2002), Monograph No. 31. Guidance on Evaluation of Reproductive Toxicity Data ISSN-0773-6347-31, Brussels, February 2002

EFSA (2017) Update: Guidance on the use of the benchmark dose approach in risk assessment. EFSA Journal 15(1):4658, 41 pp. doi:10.2903/j.efsa.2017.4658

EPA (2015), Provisional Peer-Reviewed Toxicity Values for Diethylene Glycol Monomethyl Ether, Cincinnati, https://cfpub.epa.gov/ncea/pprtv/documents/DiethyleneGlycolMonomethylEther.pdf

Edler, L., Poirier, K., Dourson, M., Kleiner, J., Mileson, B., Nordmann, H., Renwick, A., Slob, W., Walton, K., Wurtzen, G. (2002) Mathematical modelling and quantitative methods. *Food Chem Toxicol* **40**, 283-326

Leisenring, W., Ryan, L. (1992) Statistical properties of the NOAEL. *Regul Toxicol Pharmacol* **15**, 161-71.

Slob, W. (2002) Dose-response modeling of continuous endpoints. *Toxicol Sci* **66**, 298-312

WHO. (1999) Principles for the assessment of risks to human health from exposure to chemicals. Environmental Health Criteria, vol 210. WHO, IPCS. Geneva, Switzerland.

RAC's response

Noted. Thank you for the BMD analysis. The conclusion of the analysis has been considered in the Opinion.

PUBLIC ATTACHMENTS

1. DEGME CLH report 2019 - Response from GE REACH consortium 030719.pdf [Please refer to comment No. 2, 7]

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 12.08.2019 Delayed comment | UK | | MemberState | 8 |

Comment received

The UK CA does not agree that the evidence provided in the CLH dossier is supportive of classification with Repr. 1B H360D.

The justification in Section 4 (page 5) includes a list of new information providing more details on the formation and half-life of the known reproductive toxicant MAA in rats and humans. It appears that the only new information available in the current dossier (Treskelion 2017, ECETOC 2005 and Aasmoe et al. 1999) pertains to the toxicokinetics of the known reproductive toxicant MAA. Following oral administration to rats, DEGME is metabolised to produce MAA in minor quantities (1 % of the dose). The paper by Groeseneken 1989 discusses the formation and half-life of MAA in humans, however it is unclear whether this would have been considered in the original classification discussions as the paper pre-dates the original proposal.

A critical evaluation of the study by Groeseneken was not presented. However, the toxicokinetic information from humans appears to be very limited and it appears only males (n=7) were tested. It is our view that females would be more relevant to address a concern for reproductive toxicity. Since the toxicokinetic information from humans is so limited, we believe that the reproductive toxicity findings in rats should be relied upon in this assessment.

The studies presented in the CLH were generally carried out at very high doses associated with high levels of maternal toxicity (decreases in body weight and mortality). Findings at doses that far exceed the limit value should be disregarded, even if the studies did not follow test-guideline protocols. At doses below 1000 mg/kg bw findings occurred that reflected a developmental delay (variations, slightly decreased pup weight). There did not appear to be any dose-related cardiovascular, or other types of malformations at lower doses.

The assumption that developmental effects in humans would occur from lower external doses than in rats based on half-life alone is somewhat speculative. The statement 'reprotoxic effects of DEGME through the metabolite MAA cannot be excluded' is an assumption rather than a positive finding and it is our opinion that this does not provide the necessary evidence to support classification with Repr. 1B.

It is the UK opinion that the evidence provided is supportive of the current classification of Repr 2, H361d.

Dossier Submitter's Response

Thank you for your response. It is true most new information relates to the kinetics of DEGME and MAA. The studies by Groeseneken were not mentioned in the previous proposal and it seemed to us that this information was hardly or not considered at the time. With the new information it is clear MAA is formed and to what extent. The DS is of the opinion the information indicates with sufficient confidence that the half-life of MAA is

| |
|---|
| much slower in humans compared to rats. However it remains uncertain whether the formation rate is also slower in humans and there are suggestions this may be the case. However, we agree this does not clearly indicate whether effects could occur in humans at relevant dose levels as well.<br>In our response to the DEGME consortium we have presented a new BMD analyses of the most relevant animal studies for effects on development, which can also be regarded as new information (or a new analyses of existing information).<br>As also noted in our response to the consortium, the DS is of the opinion that doses above the limit dose can still be indirectly relevant as they indicate a dose response even though the effect levels at that dose may not be directly considered for classification. Doses above approximately 2400 mg/kg bw/day were not considered as the maternal toxicity became excessive. Any result up to this dose level could be used for dose-response modelling and estimating effects at the limit dose level. |
| RAC's response |
| Noted. |