Comments from the Glycol Ethers REACH consortium on the proposal for a harmonised classification and labelling of 2-(2-methoxyethoxy)ethanol (EC 203-906-6, CAS 111-77-3)

End point: Reproductive and developmental toxicity

We would like to submit comments on the proposal for the harmonised classification and labelling of 2-(2-methoxyethoxy)ethanol (diethylene glycol methyl ether, DEGME) that has been submitted by the Netherlands Competent Authority to amend the harmonised classification of the above substance. We request that the RAC take these comments into account in their discussions.

Toxicokinetics and ADME

Section 9 (p7). Triskelion study entry. Please note that the Klimisch score should read '1, reliable without restriction'.

Section 9 (p 9&10). We believe there are some errors in table 10 and that the data should be as shown in the table below. Corrected entries are shown in red[1]:

	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)

Section 9 (p11). The discussion in the classification proposal only covers the clearance issues relating to MAA elimination. The studies that are cited are those where MAA itself was given to the test animals. In the case of exposure to EGME and DEGME, the parent substance itself is not toxic and metabolism to MAA is required to elicit any toxicity (real in the case of EGME and questionable in the case of DEGME at exposures up to and including the limit dose of 1000mg/kgbw/day). Therefore the rate of formation of MAA as well as its clearance needs to be considered. For humans, whilst clearance may be slower, it is likely that rate of formation will be slower as well based on allometry considerations. This is illustrated by PBPK modelling work on EGME following inhalation exposure where exposure of pregnant rats and humans to EGME was used to calculate estimated human-equivalent no adverse effect levels based on internal dosimetry at the NOEL for developmental toxicity in rats (10 ppm). The estimated human equivalent no adverse effect level derived using average values for physiological, thermodynamic and metabolic parameters in the PBPK model was 12 ppm (Sweeney et al, 2001). This demonstrated that humans are no more sensitive than rats to a given inhalation exposure to MAA via inhalation of EGME where metabolic activation is required. If MAA is the proximate toxicant common to EGME and DEGME, then this observation would also be expected to be true for DEGME. These observations suggest that MAA

[1] This may be as a result of the data used within the CLH report having come from a draft version of the study report. The figures shown are from the final QA report of the study. However, the corrections do not alter the results significantly.

has similar potency in humans and animals and that the concerns expressed in the dossier are unfounded.

As noted above, we do not think that including data on EEA is relevant – there is sufficient data on MAA elimination kinetics not to need to use read across to ethoxyacetic acid (EAA). EAA is also cleared more readily, therefore further weakening the argument.

Reproductive toxicity (section 10.8.1 to 10.8.6)

We agree with the conclusion that, on the basis of available data, no adverse reproductive effects are seen at or below a dose of 1000mg/kgbw/day and therefore no classification is required.

Developmental toxicity (section 10.8.7 to 10.8.11)

A number of developmental toxicity studies in multiple species are available for DEGME. All are relatively old (1980's to early 1990's) and would not meet current guidelines in terms of number of animals used (insufficient) and doses administered (well above 1000mg/kgbw/day). We agree that there is data indicating possible developmental toxicity, but this is only seen to any significant extent at very high doses. We also agree that the toxic effects seen at these very high (and not relevant) doses are due to the small amounts of MAA formed during metabolism becoming significant. However, the developmental effects must be considered in the context of the associated maternal toxicity observed, particularly given that the maximum tolerated dose (MTD) was exceeded in many of these studies, and the relevance of the doses given.

Maternal toxicity

In the Hardin et al (1986) range finder study, the DEGME doses assessed were 0, 1000, 1495, 2235, 3345 and 5175mg/kg. The top dose proved fatal to 2 of the 9 animals in this group. 3345mg/kg caused a significant (18%) reduction in maternal body weight by day 21 and a significant reduction in food consumption during days 7-12 (22% reduction compared to controls). Hence the two doses used in the main study were 720 and 2165mg/kg. However, the latter still caused maternal toxicity with a statistically significant (7%) reduction in maternal body weight by day 21 and a statistically significant reduction in food consumption during days 7-12 (9% reduction compared to controls).

Yamano et al (1993) carried out range finder studies with both pregnant and non-pregnant rats. In the non-pregnant rats, urinary pH turned acidic even at the lowest doses (control pH 8.0-8.5, 125mg/kg pH 7.0-8.0, 4000mg/kg pH 5.0-6.0). It can be presumed from knowledge of the metabolism, that this was due to the main metabolite MEAA, which may well also cause metabolic acidosis, based on the evidence from another glycol ether 2-butoxyethanol (EU, 2006). The doses of 3000 and 4000mg/kg also produced statistically significantly adverse effects on blood parameters, although the trend of reducing RBC, WBC, Hb and Ht concentrations was evident from 1000-2000mg/kg onwards. Decreased haematological parameters (hemolysis) also were reported with butoxyethanol with recognition that humans are less sensitive than rats (Udden and Patton, 1994; Udden, 1994). In the main study, the doses used were 0, 200, 600 and 1800mg/kg from GD7-17. In the high dose group, there was a statistically significant reduction in maternal body weight gain (6%), food consumption (15% on day 11) and thymus weight (21%), although the authors pointed out that the former could be due to decreases in the number and weight of foetuses. It is reasonable to assume that the urine pH would have been significantly reduced in the high dose group to below 7.

In the rabbit study of Scortichini et al (1986), there were again clear signs of maternal toxicity in the top dose used (750mg/kg). Of the parameters reported, these included a marked reduction in body weight gain, which at certain periods was actually an absolute weight loss. Weight gain was only

around 50% of control animals over GD6-28, indicating that the high dose exceeded the MTD. There were also significant adverse changes to blood parameters (e.g. reduction in RBC by 7%). Anemia has been associated with altered fetal outcome in studies with pregnant rabbits (Clark et al. 1984).

Overall, at doses in excess of 1000mg/kg, there are clear signs of maternal toxicity in rats, manifest by reduced weight gain and food intake, reduced organ weight and adverse changes to blood parameters. Acidic urine is also seen and could be an indicator of blood acidosis (Yamano et al, 1993). The latter is developmentally toxic and therefore the developmental effects may be secondary to this (Khera, 1991). In rabbits, there were clear signs of maternal toxicity at the top dose tested, manifest as severely reduced body weight gain and altered blood parameters. Developmental effects associated with these changes need to be interpreted with care as they may be secondary to general maternal toxicity.

Relevance of dose

The studies available would have been carried out to old protocols which took less account of animal welfare issues and the need to limit maximum doses used. All modern test protocols have an implicit maximum oral testing dose of 1000mg/kg, specified as an accepted single dose for a limit test. These include all modern protocols for reproductive and developmental toxicity testing. Testing above these limits is normally only required if human exposure is anticipated at high doses; clearly not the case for DEGME. For this reason, it is instructive to filter the data as if it was generated to current criteria, with oral dosing limited to 1000mg/kg. In this case, the dataset would look like this:

Study [species - route]	**Test doses (n=animals/dose)**	**Results (statistically significant effects)**
Hardin et al (1986) range finder study [SD rat - gavage]	0, 1000mg/kg (n=9)	No statistically significant adverse effects seen[1]
Hardin et al (1986) full study [SD rat - gavage]	0, 720mg/kg (n=12/13)	'Total rib' malformations (15/111)[2] Variations – reduced ossification – cranial (10/111) and appendicular skeleton (6/111). Lesions also occur spontaneously in controls albeit at lower incidence. [3]
Yamano et al (1993) range finder study [Wistar rat – gavage]	0, 125, 250, 500, 1000mg/kg (n=4-6)	No adverse effects seen but no gross or detailed foetal examinations carried out.
Yamano et al (1993) full study [Wistar rat – gavage]	0, 200, 600 mg/kg (n=14)	Effects only in high dose: Reduced foetal weight (~20%). Variations: thymic remnant in the neck (unilateral) (20/98). [Reduced incidence at 1800mg/kg. (8/59).] Only one incidence in control rats. It is not unusual to have thymic remnants present in the neck of rats for up to 3 months of the postnatal periods (Kuper et al., 2016) Degree of ossification is affected.[4]
Doe (1984) [rat – subcutaneous]	0, 255, 510, 1020mg/kg (n=15) – all dose groups	No adverse effects seen, but no detailed fetal observations carried out.
Schuler et al (1984) [mouse – gavage]	*Only dose group is 4000mg/kg so study drops out of comparison using the 1000mg/kg cut off.*	
Scortichini et al (1986) [rabbit – dermal]	0, 50, 250, 750mg/kg (n=25) No filtering as this is a dermal study with all doses below 1000mg/kg.	250mg/kg: Cervical spur of vertebrae (17/194)* and delayed ossification of hyoid skull (57/194)**§. 750mg/kg: Maternal effects (reduced bw gain), GD9-11 – actually weight loss, reduced

		RBC and PVC (~7%). Fetal effects as for 250mg/kg plus mild forelimb flexure (29/68)§§, dilated renal pelvis (8/68)§, retrocaval ureter (6/68), delayed ossification of sternebrae (93/120)**§. [5] *seen at high incidence in controls. These are so common in rabbits that they are often not even reported in contemporary studies (Stump et al, 2012; DeSesso et al, 2018). In more current terminology, spurs would be considered "ossification sites" that are believed to be transient, ultimately becoming part of the lateral processes of adjacent vertebra (Chernoff and Rogers, 2004; Makris et al, 2012.) **seen at high incidence in controls. § Regarded as a variation of low to moderate concern (ECETOC, 2002). §§ seen in control rabbits.

Non-statistically significant findings that may be biologically significant.

1. One pup at 1000mg/kg showed multiple cardiovascular malformations (2% incidence). None were seen at 1495mg/kg, although the number of litters examined was lower (4 litters and 23 pups at 1495mg/kg compared to 8 litters and 38 pups at 1000mg/kg. Incidence increased at doses above this to significant levels (17% incidence at 2235mg/kg). If the finding at 1000mg/kg was 'treatment related then even with the reduced number of pups at 1495 mg/kg, an observed cardiovascular malformation would have been expected. Based on the dose response from the other three doses, an incidence rate of 4-5% would be expected or 1 pup – this was not seen, indicating the NOAEL is likely between 1000-1500mg/kg, which is consistent with the expected outcome if effects are due to the small amount of MAA formed during metabolism.
2. These consisted of 9 rudimentary cervical ribs and 6 wavy/fused, bilateral ribs, neither of which were statistically significant from the control alone. Rib anomalies (e.g., bent or wavy ribs) are common with delayed ossification and are transient and reversible. Rudimentary ribs (<35% of the length of the adjacent rib) are likely to resolve postnatally) – see DeSesso et al (2018). Even fused and branched ribs have been shown to resolve postnatally (Marr et al., 1992).
3. It was noted that one pup had a cardiovascular malformation (of the right aortic arch) at 720mg/kg with a much higher and statistically significant incidence of multiple cardiovascular malformations at 2165mg/kg.
4. It was noted that one pup had a ventricular septal defect at 600mg/kg with a much higher incidence at 1800mg/kg.
5. Single ventricular septal defect seen in low dose group – observation regarded as spontaneous and neither significantly nor biologically significant.

The Doe (1984) study did not involve any detailed fetal observations so is not complete as a developmental toxicity study, hence there are only three studies on which to comment on once the 1000mg/kgbw/day 'filter' is applied: Yamano et al (1993), Hardin et al (1986) and Scortichini et al (1986). As can be seen from the above table, once a dose ceiling is imposed, the evidence supporting significant adverse effects becomes very limited. The full study by Hardin showed only malformations in the ribs, and then only when two individual findings, neither significant on their own, were added together; one of these (wavy ribs) is regarded as a variation of low concern due to its transient nature and reversibility postnatally (DeSesso and Scialli, 2018). Note that these findings were not seen in the range finder study at 1000mg/kg. The range finder is statistically less powerful but the number of animals used was not that many fewer (9 in the range finder versus 12/13 per dose in the full study.) This throws some doubt on whether this effect is reproducible at this dose. The Yamano et al (1993)

study, using a different rat strain, showed reduced foetal weight and some slight additional variations. None of the findings were repeated across the two studies by Yamano et al (1993) and Hardin et al (1986), which again must raise questions over how repeatable these effects are at doses below 1000mg/kg. In the rabbit study (Scortichini et al, 1986), there was evidence for excessive maternal toxicity at the top dose, which exceeded the MTD, so the effects at 250mg/kg are of main interest. Observations at this dose were limited only to two variations, one of which was prevalent at a high rate in the controls; such effects are normally rated to be of low to moderate concern (ECETOC, 2002).

Human data (10.8.9, p19): The reference Karaman et al (2002) is a very brief case report. This publication postulates that there could have been exposure to either EGME or DEGME although it offers no evidence for either. Since it is purely speculation with no exposure data of any kind available and relates to the health issues of a single individual, we believe this paragraph should be deleted with a conclusion that there is no reliable human data.

Other relevant information. 10.8.10, p19: Hermsen et al (2011) should be added as useful additional data. In this study, the Zebrafish Embryotoxicity test was used to examine the developmental toxicity potential of a number of glycol ethers and their main metabolites. Hermsen et al (2011) used a novel quantitative evaluation method to assess the development of the zebrafish embryo based on specific endpoints in time, the general morphology score (GMS) system. For teratogenic effects a separate scoring list was developed and used. They assessed the acid metabolites MAA, EAA, phenoxyacetic acid, butoxyethoxyacetic acid, methoxyethoxyacetic acid and the parent glycol ethers of the first two, i.e. methoxyethanol and ethoxyethanol (EGEE). Only MAA and EAA resulted in a concentration dependent decrease in GMS. The other glycol ether metabolites did not reduce the GMS as compared to the controls up to the highest concentration that could be tested without causing general toxicity (maximum tolerated dose of 10mM). Embryos exposed to MAA and EAA showed comparable dysmorphology after exposure; several teratogenic effects were observed following exposure to these substances, among which heart, head and tail malformations (characteristics seen in mammalian developmental toxicity tests), were the most pronounced. Unlike their metabolites, the parent compounds EGME and EGEE did not show any effect on general morphology and teratogenicity. No effects were seen with the other glycol ethers. This data shows that methoxyethoxyacetic acid (the main metabolite of DEGME) does not share the developmental toxicity potential of methoxyacetic acid, an important consideration in the overall evaluation of the available data. The study is useful to rule out possible effects from either the parent compound of the main metabolite MEAA.

Comparison with the CLP criteria (Section 10.8.12)

We profoundly disagree with some of the conclusions drawn. The CLH report submitted by NL justifies using data from doses in excess of 1000mg/kgbw/day by citing the following from the CLP regulation:

"Specific developmental effects, also at relatively high doses, should not be ignored based on limit dose considerations, especially because the studies were not based on test guidelines and have a more limited power (fewer animals for example). Therefore, the developmental effects from these studies should be considered for classification (CLP criteria 3.7.2.5.7 and 3.7.2.5.9)."

We agree that there are some positive findings in the available developmental toxicity studies with DEGME but these are only significant at high doses well above the limit dose of 1000mg/kg. We do not agree with this interpretation by the author of the CLP report and believe that when the whole of the referenced paragraphs are read in their entirety, they provide explicit recognition of the fact

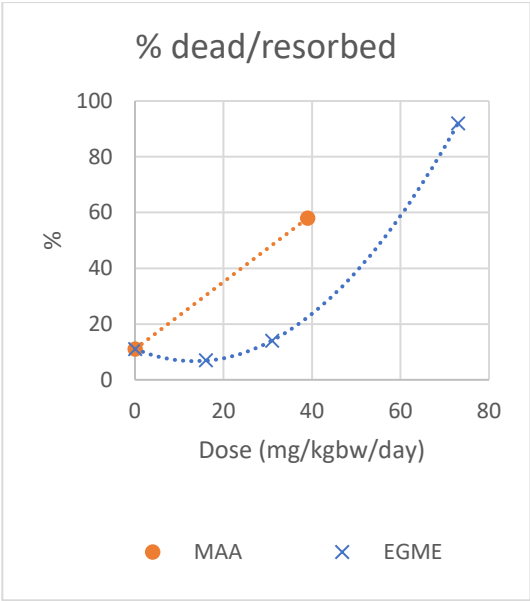
that, in this case, such data should be excluded from consideration. The whole section reads as follows:

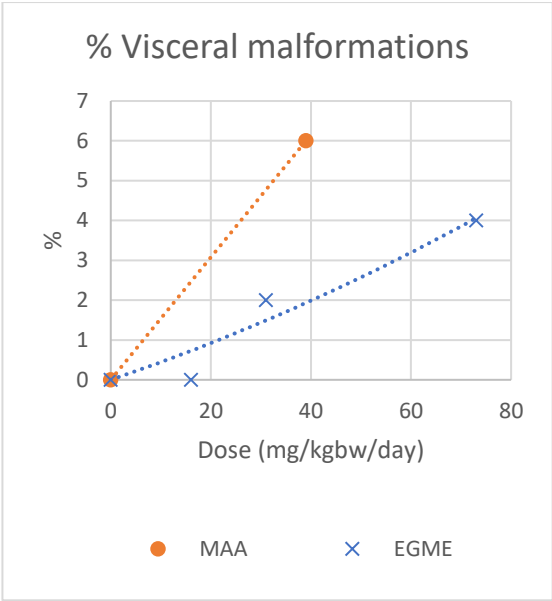
> 3.7.2.5.7. There is general agreement about the concept of a limit dose, above which the production of an adverse effect is considered to be outside the criteria which lead to classification, but not regarding the inclusion within the criteria of a specific dose as a limit dose. However, some guidelines for test methods, specify a limit dose, others qualify the limit dose with a statement that higher doses may be necessary if anticipated human exposure is sufficiently high that an adequate margin of exposure is not achieved. Also, 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.
>
> 3.7.2.5.8. In principle, adverse effects on reproduction seen only at very high dose levels in animal studies (for example doses that induce prostration, severe inappetence, excessive mortality) would not normally lead to classification, unless other information is available, e.g. toxicokinetics information indicating that humans may be more susceptible than animals, to suggest that classification is appropriate. Please also refer to the section on maternal toxicity (3.7.2.4) for further guidance in this area.
>
> 3.7.2.5.9. However, specification of the actual 'limit dose' will depend upon the test method that has been employed to provide the test results, e.g. in the OECD Test Guideline for repeated dose toxicity studies by the oral route, an upper dose of 1 000 mg/kg has been recommended as a limit dose, unless expected human response indicates the need for a higher dose level.

For this substance, based on its uses, there would be no justification for testing in excess of the limit dose. As already indicated, the main use is as an anti-icing additive in military grade jet fuels, where it is used at a concentration of ~0.15%. Exposures to such fuels during handling are very low (all enclosed handling in pipework) and it is burnt during use (no environmental exposure.) Other minor uses are again either enclosed uses (functional fluids) or industrial only (cleaners and coatings.) There are no consumer uses. There are no uses where the assessment of exposures would require the use of data from animals exposed to large doses. We therefore believe that data generated from doses in excess of this should not be given any significant weighting in a decision on classification. The author of the CLH report believes the studies have shortcomings because of inadequate number of animals to provide the statistical power required for an interpretation of the findings below 1000mg/kgbw/day. We believe that the author of the CLH report should have concluded that the studies were therefore insufficiently reliable to conclude on the developmental toxicity potential of this substance. If there are shortcomings, it is poor science to extrapolate to higher doses well above 1000mg/kg which has no relevance to potential real life hazards to humans from this substance and it would be preferable to recommend that a new study should be performed. **Industry would agree with this conclusion and to this end the lead registrant of the joint registration has submitted a new testing proposal for this substance in a recent REACH dossier update. Such a study in rats would be to the current OECD414 protocol, with testing up to the current limit dose of 1000mg/kg but with sufficient animals to be able to interpret adequately any findings below this dose level.** Furthermore, a new guideline study would require exposures throughout gestation from the time of implantation until term (e.g., GD 6-21) and would include endocrine endpoints recently added to the OECD 414 test guideline. **We therefore request that the classification process for this substance is put on hold until this testing proposal has been considered and, if approved, a definitive result is available.**

The author of the CLH report makes three points to support their proposal that classification as category 1B is justified. We do not agree that the available evidence justifies such a classification for the following reasons:

Statements in CLH report supporting classification	**Industry response**
Increased visceral 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.	To clarify, the visceral malformations referred to were only seen in rats. Effects seen that did not reach statistical significance until above the limit dose do not seem to meet the criteria of 'clear evidence' required for a category 1B classification.
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.)	We agree that the effects seen at high doses are almost certainly a consequence of formation of MAA. Whilst there may not be a study demonstrating a NOAEL for MAA, data from studies using EGME can be used. Toraason et al (1986) established a NOAEL of 25mg/kgbw/day in the rat. Taking a conservative assumption that EGME is 50% metabolized to MAA (ECETOC 2005 - see note at bottom of table), this would equate to a NOAEL for MAA of 12.5mg/kgbw/day. This provides evidence to support the case that a dose of 1000mg/kgbw/day of DEGME cannot lead to sufficient dose of MAA to exceed the NOAEL.
Furthermore, MAA has a 3.1-6.8 fold longer half-life in humans compared to rats, suggesting that developmental effects may occur in humans at lower external dose levels compared to rats.	This only takes into account metabolic clearance. This is slower in humans but it is also likely that metabolism to produce MAA in humans will occur more slowly than in rats based on allometric considerations, which will in turn likely result in a lower peak concentration.
Note The ECETOC reference is supported by Nelson et al (1989), who presented the results of studies using both MAA and EGME as the dosed substance. Results from two example and relevant lesions are shown below. These support 50% metabolization of EGME to MAA as similar incidence of lesions is seen at about half the dose for MAA compared to EGME:	





The existing data seem to fit more clearly the criteria for category 2 if there is limited evidence from experimental animals of an adverse effect on development, particularly at or below doses of 1000mg/kgbw/day. In this case the evidence is not sufficiently convincing for category 1B (the more 'adverse' effects do not reach statistical significance until well in excess of the limit dose.) There are deficiencies in the studies compared to current protocols which reduces the statistical power and therefore reduces the quality of the evidence. Developmental effects that are observed above the limit dose are seen in the presence of other signs of maternal toxicity (possibly as a consequence of general toxicity induced by the compound itself or its major metabolites).

The CLH report author has not considered the possibility of a category 2 classification. The criteria for category 2 are as follows:

> *Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.*
>
> *Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.*

Industry therefore believes that the existing data far better meets the criteria for a category 2 classification and that the existing category 2 classification for developmental toxicity is appropriate and should be retained. However, the REACH registrants are willing to perform a new rat OECD 414 developmental toxicity study to clarify the concerns expressed about the existing studies being too small to provide the statistical power to unequivocally interpret the low level incidence of findings at doses below 1000mg/kgbw/day (findings that are not statistically significant but possibly due to low statistical power as a result of too few animals). To this end, the registrants have submitted a testing proposal to perform a new guideline study.

Other comments

P21. There is a statement that "2-methoxyacetic acid was identified as an SVHC based on clear endocrine properties". This appears to be incorrect. The section containing the part of the Annex XV proposal concerning the identification of the substance as SVHC due to its endocrine disrupting properties was withdrawn by the author of this Annex XV dossier. This was motivated by discussion at the member state committee concluding that at the time of submission (2012) there remained a need for a general discussion on issues related to the identification of SVHCs under Article 57 (f) concomitant with other criteria set out in points (a) to (e) of Article 57. Criteria relating to the identification of endocrine disruptors have indeed only recently been agreed at an EU level and did not exist at the time MAA was listed as an SVHC. On the ECHA website, MAA is only indicated as an SVHC due to its reproductive toxicity. We therefore propose that this sentence is deleted.

Specific concentration limits (section 10.8.18)

The author of the CLH report did not make a calculation of the ED10, merely stating that it would be above 400mg/kgbw/day. This is correct but very conservative. It is instructive to attempt to calculate the ED10 values to show how high these are.

For the Hardin et al (1986) study (which is featured by the CLH report as the study mainly used to justify classification), for visceral effects, there are only three malformations and one variation where the ED10 is actually reached up to the maximum tested dose. For the three malformations (cardiovascular right and double aortic arch and ventricular septal defect – typical effects of MAA exposure), it is not possible to calculate an ED10 from the Hardin data as there were only two doses used and no effects were seen in the low dose. However, since both observations were also noted in the study by Yamano et al (1993), it is instructive to combine the results to estimate an ED10. The table below shows this calculation:

Combined data from Hardin and Yamano studies – visceral malformations

	Ventricular septal defect %	Double aortic arch %	Right aortic arch %
Control	0	0	0
200mg/kgbw/day	0	0	0
600mg/kgbw/day	2.4*	0	0
720mg/kgbw/day	0	0	1*
1800mg/kgbw/day	18	0	9.6
2165mg/kgbw/day	39	12	10
Calculated ED10	1460mg/kgbw/day	2000mg/kgbw/day	2200mg/kgbw/day

*one fetus. % are mean of percent fetuses per litter.

All of these ED10 values are both well above the limit dose and greatly in excess of the limit that defines low potency for reprotoxicity. The one visceral variation seen was dilated renal pelvis for which the ED10 was calculated to be 630mg/kgbw/day. (This finding was also seen in the Yamano et al (1993) study, the results of which indicated a consistent ED10 of 660mg/kgbw/day)

For skeletal effects, only two malformations were seen in the Hardin study where effects reached >10% of the foetuses, namely rudimentary cervical and wavy/fused bilateral ribs (the latter being regarded by ECETOC (2002) as of low concern). For these two observations, the ED10 values can be calculated to be in the range of 1200-1300mg/kgbw/day. For skeletal variations, the most sensitive effect was reduced ossification, for which the lowest ED10 was 670mg/kgbw/day (reduced ossification of the appendicular skeleton). Of the other variations reaching an ED10, the lowest was

960mg/kgbw/day (misaligned centra of the vertebrae). There was less repeatability between the Yamano et al (1993) and Hardin et al (1986) studies, beyond observations of reduced ossification, suggesting that the skeletal effects are more likely to be due to general rather than substance specific toxicity at the doses used.

For the fetotoxicity data, the most sensitive affected parameters were litter number and fetal body weight, for which the ED10 values were in the range 1250-1500mg/kgbw/day.

Under any measure, DEGME shows very low potency. The ED10 for all malformations exceeds 1000mg/kgbw/day and only a limited number of variations of low concern (due to their potential reversibility) show ED10 values in the range 600-1000mg/kgbw/day. Whilst there are questions regarding the robustness of the studies, collectively they show the substance to have a low potency, supported by the compelling evidence to show that the effects seen at high dose are due to the small amounts of the metabolite MAA that is produced.

We request that, whatever the outcome of the classification discussion, the RAC consider whether a specific concentration limit is appropriate for this demonstrably low potency substance. The substance clearly falls into category 3 – low potency substances. The author of the CLH report points out that the oral studies available use exposure starting relatively late during gestation (day 7) and ending earlier (day 17) than recommended for a normal OECD TG414. We do not believe that this would impact significantly on the adverse findings seen, but it further supports the industry proposal to perform a new OECD TG414 study in rats to the current protocol and wait for the outcome of this before concluding on the most appropriate classification. Since the rabbit study findings are not key to the justification behind the current classification proposal, we do not think the route of exposure of the available study is relevant. We do not think that the toxicokinetic argument made justifies not considering an SCL as the rate of clearance of MAA is only part of the picture – MAA is a metabolite and the rate of formation needs to be considered as well as its rate of clearance. As already covered, based on allometric considerations, this is likely to be slower in humans than in rats.

References

Chernoff N, Rogers JM (2004). Supernumerary ribs in developmental toxicity bioassays and in human populations: incidence and biological significance. J. Toxicol. Environ. Health B. Crit. Rev. 7: 437-449.

Clark RL, Robertson RT, Minsker DH, Cohen SM, Tocco DJ, Allen HL, James MC, Bokelman DL. (1984) Diflunisal-induced maternal anemia as a cause of teratogenicity in rabbits. Teratology 30:319–332.

DeSesso JM, Scialli AR. (2018) Bone development in laboratory mammals used in developmental toxicity studies. Birth Defects Res. 110(15):1157-1187

Doe J. (1984) Further studies on the toxicology of the glycol ethers with emphasis on rapid screening and hazard assessment. Environmental Health Perspectives; 57: 199-206.

ECETOC (2002) Guidance on Evaluation of Reproductive Toxicity data, Monograph 31, ECETOC, Brussels.

ECETOC (2005). The Toxicology of Glycol Ethers and its Relevant to Man, 4th ed, Technical Report 96, European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Feb 2005. ISSN 0773-8072-95 http://www.ecetoc.org/publication/tr-095-vol-i-the-toxicology-of-glycol-ethers-and-its-relevance-to-man-fourth-edition-volume-i/ (Accessed 31/5/19)

EU (2006). European Union Risk Assessment Report 2-butoxyethanol risk assessment https://echa.europa.eu/documents/10162/e74a38e1-b9e1-4568-92c5-615c4b56f92d (Accessed 16/06/19))

Hardin B, Goad P, Burg J. (1986) Developmental toxicity of diethylene glycol monomethyl ether (diEGME). Fundamental and Applied Toxicology; 6(3): 430-439.

Hermsen SAB, van den Brandhof EJ, van der Ven LTM, Piersma AH. (2011) Relative embryotoxicity of two classes of chemicals in a modified zebrafish embryotoxicity test and comparison with their in vivo potencies. Toxicology in Vitro 25, 745–753

Karaman, M. İ., Gürdal, M., Öztürk, M., & Kanberoğlu, H. (2002). Maternal exposure to diethylene glycol monomethyl ether: A possible role in the etiology of retrocaval ureter. Journal of Pediatric Surgery, 37(8), 1–2.

Khera KS (1991) Chemically induced alterations in maternal homeostasis and histology of conceptus: their etiologic significance in rat fetal anomalies. Teratology 44(3):259-97.

Kuper CF, van Bilsen J, Cnossen H, Houben G, Garthoff J, Wolterbeek A (2016) Development of immune organs and functioning in humans and test animals: Implications for immune intervention studies. Reprod Toxicol. 64:180-90.

Makris SL, Solomon HM, Clark R, Shiota K, Barbellion S, Buschmann J, Ema M, Fujiwara M, Grote K, Hazelden KP, Hew KW, Horimoto M, Ooshima Y, Parkinson M,Wise LD (2012). Appendix: Terminology of developmental abnormalities in common laboratory mammals (version 2). In: Hood RD, ed. Developmental and Reproductive Toxicology: A Practical Approach, 3rd Ed pp. 736-827. London, Informa Healthcare.

Marr MC, Price CJ, Myers CB, Morrissey RE (1992) Developmental stages of the CD (Sprague-Dawley) rat skeleton after maternal exposure to ethylene glycol. Teratology (2):169-81.

Nelson BK, Vorhees ICV, Scott WJ, Hastings L (1989) Effects of 2-Methoxyethanol on Fetal Development, Postnatal Behavior, and Embryonic Intracellular pH of Rats. Neurotoxicol Teratol. 11, 273-84.

Schuler RL, Hardin, BD, Niemeier RW, Booth G, Hazelden K, Piccirillo V, Smith K. (1984) Results of testing fifteen glycol ethers in a short term in vivo reproductive toxicity assay. (publication), Env Hlth Persp, 57, 141-6.

Scortichini B, John-Greene J, Quast J, Rao K. (1986) Teratologic evaluation of dermally applied diethylene glycol monomethyl ether in rabbits. Fund and Appl Toxicol; 7(1): 68-75.

Stump DG, MD Nemec, GA Parker, PS Coder, ED Sloter, B. Varsho. (2012). Significance, reliability, and interpretation of developmental and reproductive toxicity study findings, Chapter 9. In: Hood RD, ed. Developmental and Reproductive Toxicology: A Practical Approach, 3rd Ed pp. 244-316. London, Informa

Healthcare.

Sweeney LM, Tyler TR, Kirman CR, Corley RA, Reitz RH, Paustenbach DJ, Holson JF, Whorton MD, Thompson KM and Gargas ML. (2001) Proposed Occupational Exposure Limits for Select Ethylene Glycol Ethers Using PBPK Models and Monte Carlo Simulations, Toxicol Sci 62: 124-139.

Toraason M., Breitenstein M.J., Smith R.J. (1986). Ethylene glycol monomethyl ether (EGME) inhibits rat embryo ornithine decarboxylase (ODC) activity. Drug Chem Toxicol 9: 191-203.

Udden MM (1994) Hemolysis and deformability of erythrocytes exposed to butoxyacetic acid, a metabolite of 2-butoxyethanol: II. Resistance in red blood cells from humans with potential susceptibility. J Appl Toxicol. 14(2):97-102.

Udden MM, Patton CS (1994) Hemolysis and deformability of erythrocytes exposed to butoxyacetic acid, a metabolite of 2-butoxyethanol: I. Sensitivity in rats and resistance in normal humans. J Appl Toxicol. 14(2):91-6.

Yamano T, Noda T, Shimizu M, Morita S, Nagahama M. (1993) Effects of diethylene glycol monomethyl ether on pregnancy and postnatal development in rats. Arch Environ Contam Toxicol; 24(2): 228-235