Comments from the Glycol Ethers REACH consortium on the proposal for a harmonised classification and labelling of 2-butoxyethanol (EC 203-905-0, CAS 111-76-2)

End Point: Specific Target Organ Toxicity from Repeated Exposure

We would like to submit comments on the proposal for the harmonised classification and labelling for 2-butoxyethanol (or ethylene glycol butyl ether, EGBE) that has been submitted by the German Competent Authority to amend the harmonised classification of the above substance. We request that the RAC take these comments into account in their discussions. This document specifically addresses the end point of STOT from repeated exposures.

We profoundly disagree with the proposal to classify for this end point. We do not disagree that this substance caused marked haemolysis in the three commonly used animal test species of mouse, rat and rabbit. However, there is compelling evidence to show that humans and other test species such as the guinea pig are remarkably resistant to this effect. Whilst there may be a contribution towards this species difference from the established slower metabolic rate in humans and smaller proportion of BAA formed in the metabolism of EGBE, the undoubted main reason is the resistance of human erythrocytes to the haemolytic effects of BAA[1]. This conclusion is based on in vitro work using blood of numerous species, including humans, supported by the absence of haemolysis in a significant number of poisoning incidents and absence of effects seen in human volunteer studies.

The toxicology of this substance is well understood. The main and most significant substance specific toxic effect of EGBE is that it causes haemolysis, in some cases at low doses. However, it is also a highly species-specific effect and of critical importance is that rodents and rabbits are particularly susceptible to haemolysis caused by EGBE exposure whilst humans are remarkably resistant. This difference has been accepted by a wide number of regulatory bodies and taken into account when interpreting the toxicological data on the substance. For instance:

- In 1994, based on evidence to show that the acute toxicity of EGBE is better represented by data from species that are resistant to haemolysis, such as the guinea pig, the United Nations (UN) Committee of Experts deleted EGBE from its list of substances requiring special toxicity labelling. As a result, EGBE (UN2369) was not included in the Ninth Revised Edition of the UN Recommendations and since this date has not been classified as hazardous for transport (UN, 1994a, 1994b).
- The OECD under their Existing Chemicals Review Programme have reviewed EGBE on a number of occasions. It was first reviewed at SIAM 3 in 1997 with Australia as the sponsor. The substance was re-reviewed as part of the monoethylene glycol ether series at SIAM 19 in 2004 with the USA as the rapporteur. The results of the EU risk assessment were presented by France to the OECD at SIAM 23 in 2007. These reviews consistently recognised the big species differences in sensitivity between rats/mice/rabbits and humans. The 2004

[1] This is the active toxicant in all species where haemolysis occurs with the exception of dogs. The relevance of the latter is addressed later in this document.

review states “Red blood cells of humans are many-fold more resistant to toxicity from EGBE in vitro than those of rat.” (OECD, 1997, 2004, 2007)

- In 1999, The US EPA in their IRIS risk assessments of EGBE recognised that the lack of sensitivity of humans to haemolysis allowed lower assessment factors to be justified when deriving the Rfd values (US EPA, 1999). The relative differences in species response were still recognised in the update carried out in 2010 (US EPA 2010).
- In their 1999 Priority List Assessment of the substance, the Canadian EPA concluded that the available data supported at least a 10-fold difference in sensitivity between rats and humans for the haemolytic effect of butoxyacetic acid, the metabolite of EGBE (CEPA, 1999).
- In 2006, IARC recognised that rats and mice are more sensitive to EGBE-induced haemolysis than other experimental species and that humans appear to be much less sensitive (IARC, 2006)[2].
- In the 2006 EU risk assessment of EGBE, an interspecies toxicodynamic factor of 0.1 rather than the default 2.5 was used to derive the margin of safety for human risk assessment in recognition that humans are far less sensitive to the haemolytic effects of EGBE than rats, mice and rabbits (EU, 2006).

Meek (2003), then of the Existing Substances Division of Health Canada, in a publication on an initiative of the IPCS on the derivation of Chemical Specific Assessment Factors, used EGBE as an example of a substance where it is justified to reduce the toxicodynamic assessment factor from 2.5 to 0.1 due to the resistance of humans to the haemolytic effect of EGBE.

Michael Cunningham, then of the NIEHS in the USA, in his paper of 2002 used EGBE and its selective species specific haemolytic effects as an example of the differences between species and how care therefore is required in extrapolating the results from animal studies to humans.

There has been no new data published since these reviews were completed that would change the validity of these decisions.

2-butoxyethanol and haemolysis

The haemolytic effects of EGBE in sensitive species are seen following both acute and repeated exposure and can be demonstrated using in vitro approaches as well. The lack of effects in humans, including of potentially sensitive sub-populations, has been shown using in vitro approaches and also from the documentation of numerous accidental and deliberate ingestion incidents in humans along with old human volunteer studies.

The haemolysis of red blood cells is actually caused by butoxy acetic acid (BAA), the primary metabolite of EGBE (Carpenter, 1956; Ghanayem, 1987, 1989, 1993). This metabolite is produced by the alcohol and aldehyde dehydrogenase enzyme system (Ghanayem, 1987) in the liver. Rodents, guinea pigs and humans are all capable of converting EGBE to BAA. BAA appears to increase the fragility of red blood cells in some species, leading them to rupture when passing through the vascular system (Ghanayem, 1989). It also produces more haemolysis in older animals compared with younger animals. This was demonstrated to be due to the increased fragility of older red blood cells (present in higher numbers in old animals). Animals that had been bled, triggering the increased production of new red blood cells, were less susceptible to the haemolytic activity of BAA; in these

[2] Rabbits were not commented on as there was no rabbit data considered relevant to a decision on cancer hazard

animals the LD50 of EGBE was higher than animals of the same age that had not been bled, leading to the conclusion that the haemolytic activity is a critical determinant of the acute toxicity of EGBE in sensitive species (Ghanayem , 1990, 1992; Sivarao, 1995).

There is a clear species difference in susceptibility to the haemolysis caused by BAA. Acute toxicity studies in rats and rabbits show clear evidence of haemolysis; studies in guinea pigs do not. In vitro studies of red blood cell haemolysis using rodents, rabbits, guinea pigs, cats, dogs, pigs and primates (including humans) demonstrated that the red blood cells of rodents and rabbits are considerably more sensitive to this effect whereas guinea pigs and humans are significantly less sensitive (Ghanayem, 1993; Udden 1994a, 2000). Udden (1994b) also looked at potentially susceptible sub-populations of humans, including the elderly and young and those with diseases of the red blood cells (hereditary spherocytosis and sickle cell disease); none of these populations showed any susceptibility to BAA induced haemolysis.

Physiologically based pharmacokinetic models of EGBE demonstrate that it is not possible to achieve a high enough plasma concentration of BAA in humans by inhalation[3] or dermal routes[4] to trigger even a slight haemolysis of red blood cells (Corley, 1996; EU, 2006).

There are a useful number of poisoning and accidental exposure case reports that provide evidence for the haemolytic potential of EGBE in humans at very high doses (Bauer, 1992; Butera, 1996; Burkhart, 1998; Dean, 1992; Gijsenbergh, 1989; Gualtieri, 1995, 2003; Hung[5], 2010; McKinney, 2000; Rambourg-Schepens, 1988). The majority of these reports show no evidence of haemolysis following exposure to, in some cases, massive doses. In the cases where haematuria and haemoglobinuria were reported, there were other severe toxicity effects that could have been a cause of the observations. It is notable that in the publication of Dean, no haemolytic effects were noted in any of the 24 cases of accidental ingestion by children of significant amounts of EGBE. In an old volunteers study 2 men and one woman were exposed to 0.98mg/L EGBE for 2x4hr exposures with a 30 minute break in between (The Dow Chemical Co, 1955). Subsequent haematology showed no signs of haemolysis. Such an exposure in rodents or rabbits would cause profound haemolysis.

Evidence cited in the proposal to support classification

The justification for classification for this end point seems very weak and contrary to the evidence presented. There are some specific comments made that we believe can be challenged based on the scientific evidence available.

"The detailed mechanisms of action of 2-butoxyethanol and its metabolites by which severe haemolysis can be caused are not yet fully unravelled"

This statement is misleading. Mechanistic studies were performed by Udden (2003) to elucidate the mechanism of butoxyacetic acid (BAA) induced haemolysis in rat red blood cells by examining the influence of osmolarity and cation composition of the surrounding environment of rat red blood cells in vitro. Rat erythrocytes were protected from BAA-induced cell swelling and haemolysis by the addition of sucrose to the suspending media. Haemolysis and cell swelling were also reduced by

[3] Up to the saturated vapour concentration of 1160ppm.
[4] Using worse case permeability co-efficient, assuming 10% of skin surface exposed and no evaporative losses.
[5] Note that the Hung publication was not included in the CLH document – details are therefore shown in the appendix to this document.

replacing external sodium with potassium. When calcium was not present in the suspending medium haemolysis was increased; addition of as little as 0.05 mM CaCl(2) reduced haemolysis significantly while the addition of MgCl(2) had no effect. BAA-induced spherocytosis and cell fragmentation were more pronounced in the absence of calcium. The results demonstrated that the effect of calcium is to delay the onset of haemolysis. Charybdotoxin, an inhibitor of the calcium activated potassium channel, blocked the protective effect of calcium suggesting that the delay of onset of haemolysis in the presence of calcium is due to potassium loss caused by this channel. The mode of action of BAA therefore appears to be via a colloid osmotic lysis of the rat red blood cell. Haemolysis requires external sodium and is associated with calcium uptake. Udden's data suggests that BAA causes sodium and calcium to enter the cell; that calcium initially has a protective effect via the calcium activated potassium channel which facilitates the loss of potassium thereby, compensating for the osmotic effect of increased cell sodium. Calcium subsequently may have other deleterious effects through activation of proteases and externalization of phosphatidylserine in the exterior leaflet of the membrane.

It should however be pointed out that knowledge of the mode of action is not key to the decision on this end point as the data clearly establishes that humans are much more resistant to the effect than rats, rabbits and mice, and that haemolysis of human red blood cells does not occur even at doses associated with lethality in test animals.

"Consequence of a repeated or chronic exposure to this substance were never assessed in humans"

There is no evidence to suggest that chronic exposure will produce worse effects than short term exposure. In fact, the evidence points to the opposite, with sub-chronic and chronic exposures producing lesser effects than short term or single exposures. The hypothesis behind this is that older red blood cells in sensitive animals are more susceptible than newer blood cells to the haemolysing effects of BAA. This is supported by the evidence from the studies of (Ghanayem (1990, 1992) and Sivarao (1995) described previously. It is also effectively supported by the analysis of the data included in the CLH proposal. In the table below is a summary of the conclusions of the dossier. It should be noted that most of the studies are much shorter in duration than the recommendation in Annex 3.9.2.5 of the CLP regulation which states 28 or 90 day studies as the standard studies:

Duration of exposure (days) rat unless shown	STOT RE Conclusion in dossier*	Reference
Oral route		
3	Cat 2	Ghanayem (2001)
3	Cat 2	Nyska (1999)
3	Cat 2	Sleet (1991)
2-4	Cat 2	Nyska (2003)
4	Cat 2	Grant (1985)
2-4	Cat 2	Ramot (2010)
1-4	Cat 2	Ezov (2002)
1-4	Cat 2	Redlich (2004)
4	Cat 2	Shabat (2004)
7	Cat 2	Sivarao (1995
7	Cat 2	Laifenfeld (2010)

7	Cat 2	NTP (1993)
1-12	Cat 2	Ghanayem (1992)
28	Cat 2	Kenyon (2015)
42	No classification	Krasavage (1986)
90	No classification	NTP (1993)
Inhalation route		
10	Cat 1	Tyl (1984)
10	Cat 1	Mellon IIR (1952)
13 (rabbit)	Cat 2	Tyl (1984)
8-28 (dog)	Cat 2	Carpenter (1956)
30 (guinea pig)	Cat 2	Carpenter (1956)
42	Cat 2	Mellon IIR (1956)
90	Cat 2	Dodd (1983)
7-30**	Cat 2	Long (2000)
90	Cat 2	NTP (2000)
90 -730 (mouse)	No classification	NTP (2000)
180	Cat 2	NTP (2000)
365-730	No classification	NTP (2000)
730	No classification	NTP (2000)
Dermal		
9	Cat 2	Bushy Run (1989)
90	No classification ***	Wil (1993)

*this is what the conclusion would be if the animal data was used at face value without taking into account the relevance for humans. The Lewis (2006) entry is not included above as this is from a secondary source and cites other's work.

**The entry in the proposal document for this publication is misleading. These published results were from additional examinations performed on the NTP (2000) study animals, in particular an examination of 3 female rats that died during the first week of exposure to 500ppm and a 4th female rat sacrificed from this group at 30 days. The lesions were not seen after 90 days exposure, as recorded in the entry for the NTP study. The lesions were not seen in male rats or mice.

***This is the key study for the repeat dose dermal toxicity end point. This was not included in the analysis by the author of the proposal document. There was no adverse haematology at the end of this study.

We would emphasise that we do not agree with the conclusions made in this table but refer the RAC to this work by the CA which supports the case that the effect diminishes with longer exposures for reasons which are also consistent with a reversibility of the effect on ceasing of exposure. Use of the Haber rule to extrapolate NOAECs/LOAECs from shorter to longer exposure times is therefore clearly invalid for this end point. According to the guidance on application of the CLP criteria (section 3.9.5.1.1.) *Though not explicitly stated in the criteria the "... study with the longest duration should normally be used"*. If this was followed, even following the argumentation presented in the proposal dossier, no classification would be warranted based on the 90 day oral and dermal studies and the chronic inhalation study.

In conclusion, the lack of chronic or repeat dose information for humans is thus irrelevant to the issue. Data from the longest duration studies does not support classification based on the animal results alone without even taking into consideration the irrelevance of the haemolytic properties of EGBE to humans.

"In some case reports of suicide attempts, moreover, where humans consumed single oral doses of 2-butoxyethanol, e.g. in cleaning formulations, some haemolytic effects have been described in addition to more debilitating effects."

The available data from poisoning/suicide attempts shows that ingestion of even very large quantities of EGBE does not consistently produce adverse effects on the blood and when effects are reported that could be secondary to haemolysis, they are mild to moderate and found alongside severe general toxicity, so could be secondary to other effects. Haematuria was only mentioned as a finding in 3 out of the 9 publications and in these cases was associated with conservatively estimated doses of 1000mg/kg and above. It should be borne in mind that such doses are in the lethal range for animals, even those that are resistant to the haemolytic effects such as the guinea pig. The hazard is adequately reflected by the 'harmful' acute toxicity classification by the oral route.

"Another factor that needs to be taken into account when assessing the health hazard potential of 2-butoxyethanol is the high interindividual variation in permeation, absorption and elimination of 2-butoxyethanol detected in studies performed on human volunteers."

Taking into account toxicokinetic variations in ADME parameters is straying into the realms of exposure and risk assessment. Our understanding is that such issues are not taken into account in classification decisions which are based on intrinsic hazard and not variations in potential exposure. The statement is also misleading in that the only parameter where there is evidence of significant variation between humans is in dermal absorption and not in any other toxicokinetic parameter or for any other route of intake.

We do not believe that this is relevant to a decision on classification as there is sufficient information to show a qualitative difference in the effects of EGBE on the blood between rats/mice/rabbits and humans.

"The possibility exists that some humans, especially certain human subpopulations, including the elderly and those predisposed to haemolytic disorders, might be at increased risk from 2-butoxyethanol exposure, although some in vitro studies suggest the contrary (Udden, 1994; Udden, 2002)."

We believe that this statement answers its own concern. The work of Udden clearly indicates that those human sub-populations that might be at risk (the elderly, those suffering from haemolytic disorder) are as resistant to the effects of EGBE as the general population. The publication by Dean (1992) showed that children as young as 7 months old exhibit no adverse effects following exposure to EGBE. This concern can be dismissed based on the available scientific data available.

"Taken together, although humans might be less sensitive to the haemolytic effects of 2-butoxyethanol than rats, the severity of adverse effects that this substance can cause, and the variety of mammalian species which are severely affected by exposure to this chemical (including humans), and the remaining uncertainty (from the observations in dogs) whether BAA is the responsible metabolite (or the single responsible metabolite) for the haemolytic effects lead to the conclusion, that in weight of evidence a classification regarding STOT RE is warranted for 2-butoxyethanol."

We do not believe that this concluding statement follows the guidance which states *If the effects observed in animals are not considered relevant for humans then these should not be used to support classification. Similarly, if there is robust evidence that humans differ in sensitivity or susceptibility to the effect observed in the study then this should be taken into account, possibly leading to an increase or decrease in the classification assigned*. We believe there is sufficient robust evidence already presented in the CLH report and contained with the REACH registration dossier for EGBE to show that humans differ qualitatively in the sensitivity/susceptibility to the haemolytic effects of EGBE and/or its metabolite. The number of species that are sensitive is irrelevant when there is compelling and robust data to show that humans are not sensitive. The inference that humans are severely affected is an inaccurate statement that is not reflected by the scientific evidence presented in the dossier. The 'uncertainty' over whether is EGBE or its metabolite that causes the effect is also irrelevant since it is well established that exposure to EGBE results in exposure to both EGBE and BAA as a metabolite. The available data also quite clearly indicates that in all species apart from the dog BAA is the active agent causing haemolysis[6].

Application of Guidance on the Application of the CLP criteria

The guidance (v4, Jul 2017) states (3.9.2.3.2) *If there are differences in effects at the GV between studies with different duration then more weight is usually given to studies of a longer duration and (3.9.2.3.4.) If there is human data indicating no classification but there is also non-human data indicating classification then the classification is based on the non-human data unless it is shown that the human data cover the exposure range of the non-human data and that the non-human data are not relevant for humans*. We believe that there is compelling evidence to show that the non-human data is not relevant for humans for this end point. There are no reported incidences of humans suffering haemolytic effects from exposure to EGBE either in the workplace or from consumer products. The available data from poisoning/suicide attempts shows that ingestion of even very large quantities of EGBE does not consistently produce adverse effects on the blood and when these are reported, they are mild to moderate and associated with other severe general toxicity, so could be secondary in nature. It should be borne in mind that these doses are in the lethal range for animals, even those that are resistant to the haemolytic effects such as the guinea pig. This hazard is adequately reflected by the 'harmful' acute toxicity classification by the oral route. Human volunteers exposed for 8 hours by inhalation to EGBE concentrations approaching 50% of the lowest LD50 in animals and that cause severe haemolysis in such animals experienced no adverse effects on the blood.

Conclusion

Overall, we find it difficult to follow how the proposer of the classification concludes that classification for this end point is required. Whilst there is no doubt that a significant number of species are sensitive to this effect, humans are among a number of other species that are very resistant to it to the extent that it is not seen in human volunteer studies, poisoning incidents at very high doses or using in vitro studies using rat blood as a control. The classification criteria state that for classifications, findings in animals should be of relevance to human health. There is compelling

[6] The fact that the dog is apparently sensitive to haemolysis caused by EGBE rather than BAA is a curiousity that is irrelevant to the main issue which is the difference between rodents/rabbit and humans.

evidence available to show that they are not in this case and that EGBE should not be classified for haemolysis due to repeated exposure.

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Appendix

Additional information on poisoning incidents omitted from Classification Proposal

Type of data/report	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Case report	150-250mL of pure 2-butoxyethanol, corresponding to about 1.8 to 3 g/kg bw	Accidental ingestion by a 53-year man who mistook product for alcohol. Patient had already consumed alcohol	On admission to the hospital, fomepizol was given ~75 mins after ingestion in the belief that the patient had ingested ethylene glycol. The patient became comatose, suffered severe metabolic acidosis and needed treatment in intensive care using haemodialysis. This resolved in 24 hours and the patient made a full recovery. Haemolytic anaemia was not observed.	Hung (2010)