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-butoxyethanol; ethylene glycol monobutyl ether

EC Number: 203-905-0
CAS Number: 111-76-2

CLH-O-0000001412-86-226/F

Adopted
14 September 2018

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2-BUTOXYETHANOL; ETHYLENE GLYCOL MONOBUTYL ETHER

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: 2-butoxyethanol; ethylene glycol monobutyl ether
EC number: 203-905-0
CAS number: 111-76-2
Dossier submitter: Germany

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2017	United Kingdom	Glycol Ethers REACH consortium	Industry or trade association	1
Comment received				
The comments submitted here are from the Glycol Ethers REACH consortium. Please note that the submitted documents are regarded as free standing and therefore contain some duplication in content as some of the scientific evidence is relevant to more than one end point. We also attach a copy of an open access publication (5) which reviewed the acute toxicity classification of 2-butoxyethanol under the GHS system and which we believe is highly pertinent to the discussions. This document was not cited in the CLH proposal. References and file names of additional documents submitted: (1) STOT RE detailed comments. File name: "2-butoxyethanol STOT RE comments – Glycol Ethers Consortium.pdf" (2) Acute toxicity classification detailed comments. File name: "2-butoxyethanol Acute toxicity comments – Glycol Ethers Consortium.pdf" (3) Eye irritancy classification detailed comments: File name: "2-butoxyethanol Eye irritancy comments – Glycol Ethers Consortium.pdf (4) Supplementary information on eye irritancy classification. Additional information on a similar substance – referenced from document (3). File name: "F031 TEGBE Eye Irritation data 30 11 2001.pdf" (5) Boatman R, Kelsey J, Ball N (2014) Acute toxicity classification for ethylene glycol mono-n-butyl ether under the Globally Harmonized System. Regul Toxicol Pharmacol. 2014. Referenced in document (2). File name: "Boatman et al 2014.pdf". ECHA note – An attachment was submitted with the comment above. Refer to public attachment 2-butoxyethanol - comments from Glycol Ethers Consortium.zip				

Dossier Submitter's Response
The dossier submitter (DS) appreciates the comments from the Glycol Ethers REACH consortium. Detailed responses with respect to the specific endpoints addressed can be found below (nos. 3, 6, 8 and 11).
RAC's response
Thank you for your contribution.

Date	Country	Organisation	Type of Organisation	Comment number
30.11.2017	Germany	<confidential>	Company-Manufacturer	2
Comment received				
Our Company is a member of the REACH Glycolethers Consortium which already submitted comments. We totally agree with those comments and refer to them.				
Dossier Submitter's Response				
The DS appreciates the comments from the Glycol Ethers REACH consortium. Detailed responses with respect to the specific endpoints addressed can be found below.				
RAC's response				
Thank you for the comment.				

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2017	United Kingdom	Glycol Ethers REACH consortium	Industry or trade association	3
Comment received				
We do not agree with the proposed changes to the classification for this end point. Detailed comments have been submitted in a separate attachment (reference 2) explaining why we believe that the scientific evidence shows that the proposals are incorrect as regards the hazard to humans. We believe that the data indicates that the appropriate classification should be Acute tox 4 for the oral route and not classified for the inhalation and dermal routes ECHA note – An attachment was submitted with the comment above. Refer to public attachment 2-butoxyethanol - comments from Glycol Ethers Consortium.zip				
Dossier Submitter's Response				
The DS appreciates the comment from the Glycol Ethers REACH consortium. <u>Acute toxicity (oral):</u> The DS appreciates that the Glycol Ethers REACH consortium agrees with the proposed classification. <u>Acute toxicity (dermal):</u> The consortium states that rabbits are known to be specifically sensitive to butoxy acetic acid (BAA) induced haemolysis and that "the clinical findings in these studies, where reported, are consistent with haemolysis occurring". The consortium thus concluded that these "clinical and pathological findings reported from acute toxicity studies by the dermal route using the rabbit […] can be attributed as secondary to haemolysis". This interpretation is further stated to be an appropriate justification to exclude the studies with rabbits in accordance with the guidance on application of the classification and labelling criteria (section 3.1.2.3.2.), leading to a conclusion of no classification since the most recent and therefore most reliable studies in guinea pigs (the preferred species for				

this substance due to its resistance to haemolysis and therefore a closer model for human toxicity) did not show 50 % mortality when test animals were subjected to exposures of 2000 mg/kg.

The DS does not agree with this interpretation.
The DS agrees that the rabbit is to be considered the most sensitive species regarding acute dermal toxicity, as the LD_{50} values in the rat studies, for instance, all exceed the 2000 mg/kg bw threshold. *In-vitro* data may suggest that erythrocytes from humans might be less sensitive to the haemolytic effects of 2-butoxyethanol than those from rabbits. However, the severity of adverse effects (deaths and acute haemolysis) that this substance can cause, as well as the remaining uncertainties on the relevance of the available limited *in-vitro* data and whether BAA is in fact the single responsible metabolite for the haemolytic effects lead to the conclusion, that in weight of evidence a classification regarding acute dermal toxicity (Cat. 3) is justified.

For rabbits, results of the various performed studies (8 in total), in all but one case (observing a very low LD_{50}), were quite consistent: When 2-butoxyethanol was applied occlusively, calculated LD_{50} for a 24-hour application ranged from 435 to 841 mg/kg bw in 6 studies. When applied semi-occlusively, the LD_{50} was greater than 2000 mg/kg bw. Thus, the method of administration (occlusive or semi-occlusive) seems to play an important role as well, highlighting the importance of evaporation in this context. In rabbits the low(er) LD_{50} values were received after occlusive dermal application restricting substance evaporation.

It is further noted that in one (occlusive) study (Roudabush et al. 1965) - although not performed according to standard guidelines - LD_{50} values ≤ 300 mg/kg bw were identified as well when guinea pigs were used as experimental animals, also allowing for classification as Acute dermal toxicity - Category 3.

Common systemic signs of toxicity usually seen with 2-butoxyethanol in rabbits were described in the CLH report: Ataxia, laboured breathing, depression, cyanosis, sign of toxicity in the kidney, liver, thymus and spleen. These and the additional clinical signs observed in the respective studies indicate haemolysis in exposed rabbits (signs further included red fluid (possibly blood) in urine and bladder, haemoglobinuria, paleness and a dark colouration of the kidneys). The DS' evaluation is that some observed effects in rabbits indicate the occurrence of haemolytic effects that may be considered as cause of death or at least contributing to deaths. In none of the studies the study authors verified that the cause of death was in fact solely attributable to haemolysis.
Based on the assumption that the cause of death in rabbits after acute dermal exposure to 2-butoxyethanol might have been solely attributable to haemolysis and the additional assumption that BAA is the only responsible metabolite for the haemolytic effects to which rabbits are especially sensitive to (compared to humans), the DS cannot exclude all studies using rabbits as experimental animals when evaluating the endpoint acute dermal toxicity. This is especially important as the CLP Guidance (section 3.1.2.3.2) states that for acute dermal toxicity, the rat or rabbit are preferred for evaluation and "in general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested".

It is noted that human data on acute dermal toxicity of 2-butoxyethanol is not available. The consortium derived a dermal LOAEL of 1333 mg/kg for humans, which they calculated based on the proposed oral LOAEL of 400 mg/kg bw reported in the CLH, and compared the resulting value with the CLP classification criteria. The available acute toxicity data showed that for the oral route humans were as sensitive as rabbits (and

rats). The oral LD_{50} for humans was derived (with uncertainties on the dose ingested) to be in the region of 400 mg/kg bw, while the rabbit LD_{50} was 320 mg/kg.

Moreover, it has to be considered that inter-individual variation can be very high in humans. Moreover, high inter-individual variation in permeation, absorption and elimination of 2-butoxyethanol was demonstrated in studies performed on human volunteers, indicating that there is a possibility that some humans, especially certain human subpopulations, including the elderly and those predisposed to haemolytic disorders, might be at increased risk from acute 2-butoxyethanol exposure.

All in all, the DS - in accordance with the guidance on application of the classification and labelling criteria – concludes, as stated in the CLH proposal, that 2-butoxyethanol meets the criteria for classification as Acute Tox. 3, H311 according to CLP (Annex I, Part 3, Table 3.1.1 Acute toxicity Category 3 (dermal): 200 < ATE ≤ 1000 mg/kg bw), as results from studies using rabbits (and guinea pigs) revealed LD_{50} values within these thresholds.

Acute inhalation toxicity:
The consortium states that "a weight of evidence approach that takes into account the fact that the guinea pig is the best species to model the acute toxic effect of 2-butoxyethanol in humans and rabbits the worst (due to its sensitivity to haemolysis – see discussion under oral and dermal routes), and the relatively low volatility of 2-butoxyethanol (vapour pressure of ~80Pa at 20°C) should be used, leading to the conclusion from the scientific evidence that, outside of laboratory conditions, there is no acute toxicity human hazard from inhalation exposure to 2-butoxyethanol and that classification is not required for this route." Therefore, the consortium does not agree with the proposed category 3 classification cited in the submitted dossier, but supports no classification for acute toxicity by the inhalation route for vapour exposure.
The consortium further state that the "test results should be put into the context that the saturated vapour concentration at 20°C is 4.4mg/L and that the maximum that can normally be sustained under dynamic test conditions in the laboratory is 50-75% of this. Under real life conditions, even these levels cannot realistically be approached."

First, the DS notes that CLP classification is hazard based not risk based. Thus, the fact that outside of laboratory conditions, there might not be an actual inhalation exposure to sufficiently high vapour concentrations of 2-butoxyethanol is irrelevant for classification of 2-butoxyethanol under CLP.

Second, it is not clear on what basis the consortium concluded that a value of 4.4 mg/L is to be considered the maximal sustainable vapour concentration, as in a study by Tyler (1984), for instance, a higher concentration (6.4 mg/L/7h) was used and 50 % mortality was observed in guinea pigs at that concentration. Although this reference is a secondary source that cites a study report from 1943, details on exposure concentration, exposure duration, post-exposure period and LC_{50} value were reported. Accordingly, the "maximum achievable concentration of 3.9 mg/L" reported by the consortium, which would "result in a maximum theoretical dose of 187 mg/kg […] clearly well below the LOAEL derived for humans" (400 mg/kg bw), is to be considered arbitrary.

It is noted that there seem to be further acute inhalation toxicity studies, which were not cited in the CLH document, but which were presented in the RCOM process. Two studies in rats (BASF 1968 and 1978) indicating a LC_{50} between 1.1 and 5.3 mg/L/4h, one study in dogs (Dow, 1974: LC50 > 2.36 mg/L/4h), one in rabbits (Dow, 1974: LC50 ~ 2.36 mg/L/4h) and one in guinea pigs revealing a LC_{50} > 2.36 mg/L/4h (Dow, 1974). The DS

concludes that taking into account these additional data, a classification regarding acute inhalation toxicity (Cat. 3) is still to be considered justified (CLP criteria for vapour, Cat. 3: 2 > ATE ≤ 10 mg/L/4h).

All in all, there are studies available in various species indicating that classification of 2-butoxyethanol as acute inhalation toxicity - Category 3 (vapour: 2.0 < ATE ≤ 10.0 mg/L/4h) is warranted:

Rats (most sensitive species): LC50(females and old animals) = 2.2 mg/L/4h;
LC50(males) = 2.37 mg/L/4h
Mice: LC50 = 4.12 mg/L/4h
Guinea pigs: LC50 = 6.4 mg/L/7h (corresponding to 7.65 mg/L/4h, Habers rule)
Dogs: LC50 > 2.36 mg/L/4h
Rabbits: LC50 = 2.36 mg/L/4h.

Taking into account that human data indicate that inter-individual variation in permeation, absorption and elimination of 2-butoxyethanol is very high, further suggests that there is a possibility that some humans, especially certain human subpopulations, including the elderly and those predisposed to haemolytic disorders, might be at increased risk from acute 2-butoxyethanol exposure. Hence, appropriate classification is considered particularly necessary.

RAC's response

Thank you for your contribution.

Date	Country	Organisation	Type of Organisation	Comment number
08.11.2017	France		MemberState	4

Comment received

Acute toxicity – Oral route
FR agrees with the classification proposal. However, some comments arise from the CLH report.
Page 54, in the beginning of the justification for ATE paragraph, you mention "relevant and acceptable studies". However, in any part of the document, the reliability and the acceptability of studies is discussed. We understand that it could be the studies with the lowest LD50 that are mentioned here, but in this case, it has nothing to do with the relevance of the study. Moreover, in the same paragraph, based on the LD50 mentioned, a conclusion is made on the sensitivity of the different species of the tests. This conclusion seems hazardous to us, because it is based (apparently) on the lowest LD50 only, and there is only one study with rabbit, and 2 with guinea pig.
In the same way, page 52-53, it is said that recent studies have given results between 1000 and 2600 mg/kg in rat, without any precision on which studies are mentioned.

Finally, FR disagrees with the choice of ATE. For 2-butoxyethanol, there is a wide database, with not less than 16 studies on acute toxicity by oral route. The guidance stated that "The acute toxicity estimate (ATE) for the classification of a substance in a mixture is derived using the LD50/LC50 where available", and that "an available LD50/LC50 is an ATE at first stage". Consequently, FR is of the opinion that a LD50 chosen from the database should have been used as an ATE with an appropriate justification.

Acute toxicity – dermal route
FR agrees with the classification proposal. However, FR disagrees with the choice of the

ATE. For 2-butoxyethanol, there is a wide database, with not less than 15 studies on acute toxicity by dermal route. The guidance stated that "The acute toxicity estimate (ATE) for the classification of a substance in a mixture is derived using the LD50/LC50 where available", and that "an available LD50/LC50 is an ATE at first stage". Consequently, FR is of the opinion that a LD50 chosen from the database should have been used as an ATE with an appropriate justification.

Acute toxicity – inhalation route
FR agrees with the classification proposal. However, like for oral and dermal routes, FR disagrees with the choice of ATE. There is again a wide database. The guidance stated that "The acute toxicity estimate (ATE) for the classification of a substance in a mixture is derived using the LD50/LC50 where available", and that "an available LD50/LC50 is an ATE at first stage". Consequently, FR is of the opinion that a LC50 chosen from the database should have been used as ATE with an appropriate justification.

Dossier Submitter's Response

The DS appreciates the comment from FR.

Acute toxicity – Oral route:
The DS agrees that it should have been clarified to which studies this sentence refers to. However, in Table 9, all "relevant studies" are cited and it is further clarified which studies were performed according/similar to any validated guideline and are thus acceptable/ reliable. Also the studies mentioned on pages 52-53, saying that recent studies have given results between 1000 and 2600 mg/kg in rat, refer to the studies mentioned in Table 9 (Eastman Kodak, 1981; Dow Chemical, 1981; Bushy Run, 1980). However, the results reported were between 1000 and 2420 mg/kg bw for rats not 1000 – 2600 mg/kg bw.

The conclusion that the rabbit is the most sensitive species is in fact based on the (lowest) LD_{50} values obtained in the various studies. It is debatable whether species sensitivity can be extrapolated from those values. There are 16 studies mentioned in table 9: 9 studies in rats, 4 studies in mice, 1 in rabbits and 2 in guinea pigs. The study performed in rabbits, as well as several studies in rats (5 studies), 1 study in mice and 1 study in guinea pigs were conducted similar to OECD TG 401 and are thus considered reliable. The DS considers that comparison of LD_{50} values obtained from reliable studies is an appropriate method for determining species sensitivity with respect to acute oral toxicity. However, discussion in RAC is welcome.

The DS considers the oral LD_{50} in rabbits (320 mg/kg bw) as appropriate ATE value for classification of 2-butoxyethanol as Acute Tox. 4. The DS further highlighted that even considering results for other species, classification of 2-butoxyethanol as Acute Tox. 4 is considered to be justified. According to CLP, Annex I, Table 3.1.2. the converted acute toxicity point estimate for oral administration classified in the hazard Category 4 (based on the lowest LD_{50} in rabbits) is 500 mg/kg bw (see Note 1: 'These values are designed to be used in the calculation of a mixture based on its components and do not represent test results.').

Acute toxicity – dermal route:
The DS appreciates that FR agrees with the proposed classification.
The DS considers the dermal LD_{50} in rabbits (435 mg/kg bw) as appropriate ATE value for classification of 2-butoxyethanol as Acute Tox. 3.
According to CLP, Annex I, Table 3.1.2. the converted acute toxicity point estimate for dermal administration classified in the hazard Category 3 (based on the lowest LD_{50} in

rabbits) is 300 mg/kg bw (see Note 1: 'These values are designed to be used in the calculation of a mixture based on its components and do not represent test results.').

Acute toxicity – inhalation route:
The DS appreciates that FR agrees with the proposed classification.
The DS considers the inhalation LC_{50} in female rats (2.2 mg/L/4h) as appropriate ATE value for classification of 2-butoxyethanol as Acute Tox. 3. Nevertheless, the DS highlighted that even considering the LC_{50} values in male rats or other species justify classification of 2-butoxyethanol as Acute Tox. 3.
According to CLP, Annex I, Table 3.1.2. the converted acute toxicity point estimate for inhalative administration classified in the hazard Category 3 (based on the lowest LC_{50} in female rats) is 3 mg/L/4h (see Note 1: 'These values are designed to be used in the calculation of a mixture based on its components and do not represent test results.').

RAC's response

Thank you for your contribution.

Date	Country	Organisation	Type of Organisation	Comment number
30.11.2017	Germany	<confidential>	Company-Manufacturer	5

Comment received

We do not agree with the proposed changes to the classification for this end point. We believe that the data indicates that the appropriate classification should be Acute tox 4 for the oral route and not classified for the inhalation and dermal routes. Rat, mouse, rabbit, hamster and baboon are species which are sensitive to butoxy acetic acid (BAA) induced haemolysis, whereas human, guinea pig, dog and cat are species resistant to BAA induced haemolysis. BAA is the primary metabolite of EGBE. Because of the similarity in sensitivity to haemolysis we suggest to use the LD50-values of the guinea pig as basis for classification. A classification based on the LD50-values of rat, mouse and rabbit would lead to an overestimation of the hazard.

Dossier Submitter's Response

The DS appreciates the comment.
Detailed responses can be found in response to comment no. 3.

RAC's response

Thank you for your contribution.

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2017	United Kingdom	Glycol Ethers REACH consortium	Industry or trade association	6

Comment received

We have no specific comments on this end point and support retention of the current classification of Category 2.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment 2-butoxyethanol - comments from Glycol Ethers Consortium.zip

Dossier Submitter's Response

The DS appreciates the comment by the Glycol Ethers REACH consortium and their agreement with the proposed classification.

RAC's response

Thank you for your agreement.

Date	Country	Organisation	Type of Organisation	Comment number
08.11.2017	France		MemberState	7
Comment received				
Even if this is a borderline case, FR agrees with the classification proposals.				
Dossier Submitter's Response				
The DS appreciates the comment by FR and their agreement with the proposed classification.				
RAC's response				
Thank you for your agreement.				

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2017	United Kingdom	Glycol Ethers REACH consortium	Industry or trade association	8
Comment received				
We do not agree with the proposed changes to the classification for this end point. The conclusion is out of line with previous EU classification decisions based on the same data and criteria for reversibility of effects. Detailed comments have been submitted in separate attachments (references 3,4) explaining why we believe that the scientific evidence shows that the proposal is incorrect as regards the hazard to humans. We believe that the data indicates that the current classification of Eye irritancy cat 2 is appropriate and should be retained but that a specific concentration limit of 20% for mixtures is justified based on the available evidence ECHA note – An attachment was submitted with the comment above. Refer to public attachment 2-butoxyethanol - comments from Glycol Ethers Consortium.zip				
Dossier Submitter's Response				
The DS appreciates the comment by the Glycol Ethers REACH consortium. The DS further agrees that the reliable *in vivo* studies mentioned in section 9.5.1 only describe effects which are in accordance with a classification of 2-butoxyethanol as Eye Irrit. 2. However, in two studies – both performed in compliance with GLP and according to the validated OECD TG 405 - some crucial effects were not reversible within 21 days, specifically chemosis (1/3 animals still showed a score of 1.0 at day 21: ECETOC, 1998; 1/6 animals still showed a score of 2.0 at day 14 and was sacrificed thereafter: Safepharm, 1994), redness (2/3 animals still showed a score of 1.0 at day 21: ECETOC, 1998; 1/6 animals still showed a score of 2.0 at day 14 and was sacrificed after observation: Safepharm, 1994), iritis (1/6 animals still showed a score of 1.0 at day 14 and was sacrificed after observation: Safepharm, 1994) and cornea opacity (2/6 animals still showed a score of 2.0 (vascularisation) after 14 (then sacrificed) and 21 days, respectively; Safepharm, 1994 b). A further irreversible effect was ectropion in some animals from 72 h, which was not reversible in 1/6 animal within 21 days. The consortium states that "the lesions were disappearing and based on the rate of decline in their severity, it is reasonable to hypothesise that the effects seen in this last animal would have disappeared within a further 1 week." The DS considers this statement speculative as this assumption cannot be validated. Moreover, even if the effect would have resolved within another week, the CLP criteria				

always refer to an observation period of 21 days in which the symptoms have to resolve to be able to classify the respective substance into Cat. 2.

It is correct that in the study by ECETOC no details with respect to washing of the substance was reported and washing is, thus, not assumed to have been conducted. In the study by Safepharm, it is reported that eyes were not washed, which might have contributed to the reported long-lasting effects. However, although washing of eyes is permitted in OECD TG 405, it is not a prerequisite. At 24 hours a washout may rather be used if considered appropriate. Thus, while omitting washing might overestimate the severity of effects, using a washing step or applying diluted concentrations of the test substance might clearly result in underestimation of effects.

It is further noted that *in vivo* results using the test substance always have higher priority than *in vitro* results or results from studies using similar substances.

Taken together, the CLP guidance clearly state that "a substance is considered to cause irreversible effects on the eye (Category 1) if, when applied to the eye of an animal, it produces:

- at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse **or have not fully reversed within an observation period of normally 21 days**; **and/or**
- at least in 2 of 3 tested animals, a positive response of: corneal opacity ≥ 3 and/or iritis > 1.5 (calculated as the mean score following grading at 24, 48, 72 hours after installation of the test material)

While "a substance is considered to cause reversible effects on the eye (Category 2) if, when applied to the eye of an animal, it produces:

- at least in 2 of 3 tested animals, a positive response of: corneal opacity ≥ 1, and/or iritis ≥ 1, and/or conjunctival redness ≥ 2, and/or conjunctival oedema (chemosis) ≥ 2 (calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material), and **which fully reverses within an observation period of 21 days**."

As the results of the two reliable studies by Safepharm and ECETOC demonstrated adverse effects on several measured endpoints (chemosis, redness/conjunctivae, iritis and cornea opacity) that were not reversible within 21 days after application, classification of 2-butoxyethanol as Eye Dam. 1 is considered justified.

RAC's response

Thank you for your contribution.
In the study reported by ECETOC (1998), chemosis effects (1/3 animals) and conjunctivae effects (2/3 animals) were not fully reversible within 21 days. In the study conducted by Safepharm laboratories (1994b) one animal (1/6) showed a cornea opacity score of 2.0 (vascularisation) at day 21. Moreover, ectropion was seen in some animals from 72 h, it was not reversible in 1 animal at day 21; one rabbit showed signs of distress and had to be sacrificed at day 14.
Therefore results of studies by ECETOC (1998) and Safepharm laboratories (1994b) (performed according to OECD TG 405 protocol, GLP compliant, relevant and reliable) fulfil CLP criteria for classification of 2-butoxyethanol for irreversible effects on the eye, category 1 if at least in one animal effects on iris that have not fully reversed within an observation period of normally 21 days.

Date	Country	Organisation	Type of Organisation	Comment number
08.11.2017	France		MemberState	9
Comment received				
Even if this is a borderline case, FR agrees with the classification proposals.				
Dossier Submitter's Response				
The DS appreciates the comment by FR and their agreement with the proposed classification and agrees that this case might be considered as borderline.				
RAC's response				
Thank you for your contribution.				

Date	Country	Organisation	Type of Organisation	Comment number
30.11.2017	Germany	<confidential>	Company-Manufacturer	10
Comment received				
We believe that the data indicates that the current classification of Eye irritancy cat 2 is appropriate and should be retained but that a specific concentration limit of 20% is justified based on the available evidence. Three studies are available that were continued for 21 days sufficient to observe reversibility over the period stipulated in current guidelines. The crucial difference between these studies is that in the BASF study, the eyes were washed after 24 hours whereas they were not in the other two. The BASF study showed full reversibility, whereas in the two other studies one animal had some residual symptoms at the end of the 21 day period. The eye wash option was introduced into the guideline in order to better represent the human situation and therefore studies using this approach should be given a high weighting. Therefore we suggest maintaining the current classification of Eye irritancy cat 2.				
Dossier Submitter's Response				
The DS appreciates the comment. A detailed response can be found under comment no. 8.				
RAC's response				
Thank you for your contribution. A detailed response can be found under comment no. 8.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2017	United Kingdom	Glycol Ethers REACH consortium	Industry or trade association	11
Comment received				
We do not agree with the proposed changes to the classification for this end point. Detailed comments have been submitted in a separate attachment (1) explaining why we believe that there is compelling scientific evidence to show that the effects used to justify the proposal are not relevant to humans and that this has been accepted by all regulatory bodies that have review the toxicity of this substance in the past, including the EU on multiple occasions. We believe that classification for this end point is completely inappropriate.				

ECHA note – An attachment was submitted with the comment above. Refer to public attachment 2-butoxyethanol - comments from Glycol Ethers Consortium.zip

Dossier Submitter's Response

The DS appreciates the comment by the Glycol Ethers REACH Consortium, however, the DS does not agree with the interpretation that classification for this end point is inappropriate. Discussion in RAC is welcome.

The consortium states: "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. 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 DS appreciates that the consortium agrees that 2-butoxyethanol causes marked haemolysis in various animal species (e.g. mice, rats, rabbits). The DS further notes that results of some *in vitro* studies, as well as predictions generated by a PBPK model indicate that humans might be less sensitive to the haemolytic effects of 2-butoxyethanol compared to other species such as rats and mice. Mechanistic *in vitro* studies indicate that the metabolite BAA is likely involved in and may be the main responsible agent for the haematotoxicity caused by exposure to 2-butoxyethanol in most mammals. The proposed differences between species were suggested to be due to the slower metabolic rate and the lower percentages of 2-butoxyethanol being converted to BAA in humans e.g. versus rats, as well as the lower susceptibility of human erythrocytes to BAA effects *in vitro* compared to rat erythrocytes. However, other mammalian species such as dogs were shown to be adversely affected by 2-butoxyethanol directly, leading to severe haemolysis. The dogs, however, were not affected by exposure to BAA. This indicates that haemolysis due to 2-butoxyethanol exposure cannot only be attributable to BAA action, but rather indicates that there might be another – not yet understood – mechanism also leading to severe haemolytic effects. This finding contradicts the proposal by the consortium that haemolysis of red blood cells is only caused by BAA.

The consortium further states that "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."
This conclusion is based on *in vitro* studies using blood cells of numerous species, including humans. Udden (1994 a and b, 2002) incubated red blood cells (RBC) from rats or healthy young and older humans and from patients with hereditary spherocytosis and sickle cell disease, respectively, with BAA. No changes in deformability due to treatment with BAA were detected in the human cells of different origin, whereas rat erythrocytes showed evidence of mild haemolysis, indicating differences in susceptibility between rats and humans in this test. Ghanayem and Sullivan (1993) similarly exposed RBCs to BAA and found a species-dependent effect. However, in this context the DS highlights that *in vitro* studies do not necessarily reflect *in vivo* conditions but rather can be used as an indicator for potential effects *in vivo*. Thus, caution is required when extrapolating from *in vitro* studies, particularly with respect to species (or human sub-population) comparisons, and especially regarding quantification of *in vivo* susceptibility. Quantification of species-

differences even on the basis of in vivo studies is always associated with considerable uncertainty.

The DS agrees that there is "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; Hung5, 2010; McKinney, 2000; Rambourg-Schepens, 1988)." Although it is noted that in some of these reports no evidence of haemolysis following exposure was reported, in other cases severe haemolytic effects were observed. It needs to be kept in mind that these are case studies, which means that not necessarily all parameters that might have been of interest were identified/measured. Moreover, human subjects were exposed to 2-butoxyethanol acutely, only once. Consequences of a repeated or chronic exposure to this substance were never assessed in humans.
The consortium, on the contrary, claims that "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." The consortium further state: "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."
The DS notes that the assumption that haemolytic effects due to 2-butoxyethanol exposure diminish over time is based on the following results in rats, whereas no evidence in humans is available:
In the study by Ghanayem et al. (1990), male rats were treated with different concentrations of 2-butoxyethanol by repeated gavage administration and haematology profiles were analysed using different methods. Profiles from the impedance-based analyser showed an early dose- and time-dependent increase in haematocrit (HCT) and mean cell volume (MCV). In contrast, analysis of the same blood samples using the laser-based analyser showed a dose- and time-dependent decrease in HCT with little or no change in MCV, highlighting that methodological differences in study design can result in marked differences in results. Results further indicated that 2-butoxyethanol might cause acute swelling of erythrocytes followed by haemolysis. The authors however did not unravel whether 2-butoxyethanol itself or a metabolite such as BAA (or both) is causing this effect.
In follow-up studies, Ghanayem (1992) treated rats with 2-butoxyethanol daily for 1 to 3 consecutive days and found a time-dependent increase in the haemolysis of erythrocytes. However, when daily treatment was continued beyond 3 days (until day 12), the authors stated that the number of erythrocytes began to rebound after 6 days and approached pre-treatment levels within 12 days despite continued daily exposure, suggesting development of tolerance to the haemolytic effect of 2-butoxyethanol. In a challenge experiment, a significant decline in the sensitivity of 2-butoxyethanol-pre-treated/recovered rats compared to vehicle-pre-treated rats was observed. The DS' view is that a part of these results might be attributable to compensation mechanisms due to increased erythropoiesis and considers it uncertain whether a true tolerance mechanism (resulting in a lower degree of haemolysis) was detected.

In vitro incubation of blood obtained from the bled/recovered animals with BAA indicated that erythrocytes from these animals were significantly less sensitive to BAA than those obtained from control rats. This observation was interpreted by the authors that young

erythrocytes were less sensitive to BAA and found it unlikely that tolerance caused a modification of 2-butoxyethanol metabolism. However, there were no specific studies on metabolic capacity or haemolytic responses of the fraction of 'young' immature erythrocytes. Effects of 2-butoxyethanol per se or other metabolites of this substance, which might be able to cause haemolysis as well (see results in dogs) were not analysed.

The other study mentioned by the consortium is the publication by Sivarao (1995). Here, the authors performed a challenge experiment with female rats. All rats receiving an initial 'protective' dose of 2-butoxyethanol were able to survive the challenge (lethal dose of 2-butoxyethanol), in contrast to the death of those receiving the lethal dose alone. The authors also found that increasing the time between pre-treatment and challenge decreases the 'auto-protection ability'. It was suggested that this might be due to aging of the red blood cells.
According to the authors administration of pyrazole to inhibit metabolism of 2-butoxyethanol to BAA abolished the auto-protection, indicating that BAA is responsible for this auto-protection mechanism. However, these results do not demonstrate that BAA is the only metabolite of 2-butoxyethanol by which haemolysis might be induced. On the contrary, although no specific details were reported in the publication, the authors stated that parallel treatment of rats with 2-butoxyethanol and pyrazole resulted in "profound ataxia that lasted several hours more than that observed in control rats", suggesting that even without the postulated conversion of 2-butoxyethanol to BAA severe adverse effects occur. HCT values, however, were not decreased 24 h after 2-butoxyethanol and pyrazole treatment when compared to rats not pre-treated with pyrazole. Nevertheless, as HCT values were not measured during the phase of ataxia, one can only conclude that haemolytic effects due to BAA action were more severe and/or lasted longer than haemolytic effects due to 2-butoxyethanol + pyrazole exposure (inhibiting the conversion to BAA); it does, however, not demonstrate that other metabolites or 2-butoxyethanol itself are not able to induce haemolysis but rather on the contrary indicates that also without conversion of 2-butoxyethanol to BAA adverse effects can occur.

The DS agrees with the consortium that most of the studies used for classification 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. However, the DS highlights that also studies analysing effects of long-term studies (28-days oral exposure; 6-weeks, 90-days and 14-weeks inhalation exposure), revealed effects that warrant classification of 2-butoxyethanol as STOT RE 2, including body weight loss, mortality, lethargy, haemoglobinuria, significant reduction in RBC counts, significant reductions in Hb concentrations and HCT, significant increase in reticulocyte, lymphocyte and monocyte counts, significant increases in MCV and MHC, increased erythrocyte fragility, increased organ weights and histopathological lesions in various organs (Carpenter et al., 1956; Dodd et al., 1983; NTP, 2000; Kenyon et al., 2015).
The DS further agrees with the consortium that chronic exposure to 2-butoxyethanol might produce no worse effects than short term exposure due to compensation mechanisms (responsive increases in erythropoiesis) in response to the haemolytic anaemia or a potential decrease in susceptibility of newly formed erythrocytes after the occurrence of the anaemia. Nevertheless, the DS is of the opinion that the Haber's rule still can be applied here, especially in view of the above mentioned findings in the long-term studies. Discussion in RAC in this respect, however, is welcome.

The consortium also does not agree that inter-individual variation, which was demonstrated e.g. for ADME in humans, should not be "taken into account in classification decisions that are based on intrinsic hazard and not variations in potential exposure." This statement, however, is correct with regards to the exposure conditions. Variations in exposure or specific exposure scenarios are in fact not to be taken into account when

classifying substances. However, ADME behaviour and inter-individual variation in this behaviour does not reflect exposure but rather differences in susceptibility with respect to the intrinsic hazard of 2-butoxyethanol. Especially as the consortium suggests that humans are of lower susceptibility than other mammals such as rats or mice, the inter-individual variability with respect to individual human subjects but also to different human sub-populations plays a very important role. If those species-differences were quantified as done by the consortium, usually mean values (of susceptibility) are used. If, however, the inter-individual variation is very high, specifically the more susceptible subjects are not sufficiently taken into account in this equation. The quantified differences between species or sub-populations, thus, would be an underestimation of actual differences for some individuals or sub-populations. The DS, hence, concludes that the high inter-individual variation in permeation, absorption and elimination of 2-butoxyethanol detected in studies performed on human volunteers are in fact of high relevance in the context of classification of 2-butoxyethanol.

The consortium concluded that "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. […] 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."

Referring to the above reported statements, the DS in contrast concludes that differences in susceptibility between the tested species are not validated due to the lack of comparative *in vivo* data following repeated exposure (note that similar adverse effects were seen in acute toxicity for the oral route) and thus are rather speculative. Moreover quantification of species differences based on *in vitro* data should be omitted. Even if *in vivo* data indicated species-differences, quantification of those would be associated with a considerable level of uncertainty. All in all, several species including mice, rats, rabbits and dogs were clearly shown to be adversely affected by 2-butoxyethanol exposure. Moreover, the fact that while dogs were affected by 2-butoxyethanol directly, leading to severe haemolysis, they were not affected by exposure to BAA, is of high importance. This indicates that haemolysis due to 2-butoxyethanol exposure cannot only be attributable to BAA action, but rather suggests that there might be another – not yet understood – mechanism also leading to severe haemolytic effects, contradicting the statements of the consortium. These results cannot be neglected in the context of classification, and – on the contrary to the opinion of the consortium – are very well of relevance for the proposed classification.
The available data from poisoning/suicide attempts further show that ingestion of large quantities of 2-butoxyethanol does not necessarily produce adverse effects on the blood. Nevertheless, such adverse haematological effects were observed in several cases, indicating that in humans the inter-individual susceptibility to 2-butoxyethanol exposure is in fact high. As no repeated dose data in humans are available, potential outcomes of such studies remain speculative.

Taken together, as stated in the CLH proposal, although there are indications from *in vitro* testing that human cells 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), as well as the remaining uncertainty from the observations in dogs whether BAA is the only responsible metabolite for the haemolytic effects lead to the conclusion, that in weight of evidence a classification regarding STOT RE (Cat. 2) is warranted for 2-butoxyethanol.
RAC's response
Thank you for your contribution.

Date	Country	Organisation	Type of Organisation	Comment number
08.11.2017	France		MemberState	12
Comment received				
Considering the database, FR is of the opinion that studies with very short duration should have not been taken into account for STOT RE. Studies with longer exposure to the substance lead to a less severe classification or no classification at all. This should have been discussed.				
Dossier Submitter's Response				
We appreciate the comment by FR and welcome a discussion on which studies with respect to study/exposure duration should be included, when evaluating haemolytic effects. The DS, however, highlights, as mentioned in detail in the response to comment no. 11, that also studies analysing effects of a 28-day oral, a 6-week, a 90-day, and a 14-week inhalation exposure, respectively, revealed effects that warrant classification of 2-butoxyethanol as STOT RE 2, including body weight loss, mortality, lethargy, haemoglobinuria, significant reduction in RBC counts, significant reductions in Hb concentrations and HCT, significant increase in reticulocyte, lymphocyte and monocyte counts, significant increase MCV and MHC, increased erythrocyte fragility, increased organ weights and histopathological lesions in various organs (Carpenter et al., 1956; Dodd et al., 1983; NTP, 2000; Kenyon et al., 2015).				
RAC's response				
Thank you for your contribution.				

Date	Country	Organisation	Type of Organisation	Comment number
30.11.2017	Germany	<confidential>	Company-Manufacturer	13
Comment received				
We disagree with the proposal to classify for this endpoint. 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. The classification criteria state that for classifications, findings in animals should be of relevance to human health. There is compelling evidence available to show that they are not in this case and that EGBE should not be classified for haemolysis due to repeated exposure.				
Dossier Submitter's Response				
We appreciate the comment. A detailed response can be found under comment no. 11.				
RAC's response				
Thank you for your contribution.				

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2-BUTOXYETHANOL; ETHYLENE GLYCOL MONOBUTYL ETHER

PUBLIC ATTACHMENTS

1. 2-butoxyethanol - comments from Glycol Ethers Consortium.zip [Please refer to comment No. 1, 3, 6, 8, 11]