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

End point: Eye irritancy

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 eye irritancy and contains what we believe is a more appropriate interpretation of the data on 2-butoxyethanol as it pertains to the hazard presented to humans.

Introduction

The harmonised classification of 2-butoxyethanol (EGBE) has been reviewed at EU level on two occasions and the appropriate eye irritancy classification was discussed at both. A proposal was made in 1999 by France (ECBI/35/99) for a change in classification. Amongst other classifications, it was proposed to classify the substance R41 but the existing classification of R36 was retained once new data generated to the most recent OECD protocol was taken into account (BASF, 2000). The classification was again reviewed when EGBE was examined under the EU Existing Substances regulation (report published in 2006) when, again, the R36 risk phrase and classification was felt to best represent the hazard to humans.

There is no new data available since the EU risk assessment was performed.

We recognise that the criteria for classification for eye hazards have changed with the implementation of the CLP regulations and that these are somewhat stricter than those of the old Dangerous Substances Directive. However, the main issue for EGBE is not the acute severity of the lesions seen, which do not reach the threshold for classification for irreversible eye damage under either the DSD or CLP criteria, but rather the length of time the lesions take to disappear and the criteria for this are the same under both the old Dangerous Substances Directive 67/548 and the replacement Regulation 1272/2008, therefore, there is no formal justification to discuss this endpoint again.

In vivo studies available for EGBE

There are a large number of eye irritation studies available for this substance that range in quality from Klimisch 1 GLP studies according to modern protocols to very old studies that are of limited value. The interpretation of these needs to be weighted in terms of the information they contain and the relevance of the protocols followed in terms of their applicability to the human situation. It should be emphasised that whilst the regulatory regime has changed, there is no new data available now that was not considered in 1999 and 2006.

The data available falls into three categories:

- Studies where data available in sufficient detail at individual animal level to enable comparison with CLP criteria (Table 1).
- Studies where data available but not at individual animal level to enable full comparison with CLP criteria (Table 2).

- Studies on diluted substance. There is also supporting information on dilutions of a similar substance that demonstrate how eye irritancy properties fade and disappear with increasing dilution (Table 3).

The studies where data is available in sufficient detail at the individual animal level to allow an unambiguous comparison with the classification criteria should be given the highest weighting (table 1). When the harmonised classification of EGBE was reviewed in 1999 and 2006, the highest weighting was given to the GLP BASF (2000) study as this was the most recent and carried out to the most recent guideline. The key difference with the other studies with information available at this level of detail is that washing of the eyes was carried out after 24 hours as this approach more accurately reflects the lachrymatory response of humans (a response lacking in rabbits). This study showed acute effects that warranted a classification as category 2 and all lesions resolved within 21 days. The Safepharm and Huntingdon studies showed similar acute effects (similar MMAS scores) but did not use eye washing after 24 hours. The acute effects in the ECETOC study (also without eye washing) seemed somewhat more severe (higher MMAS score) and for the iritis effects were on the border of classification as category 1. The Huntingdon study was only continued for 7 days therefore provides no information on lesion resolution. The ECETOC study showed the lesions to be resolving but conjunctival redness persisted in one animal at the end of the 21 day observation period. However, 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 Safepharm study used 6 animals twice the normal number now recommended and which needs to be taken into account when evaluating the results. Effects resolved in all but one animal where corneal effects persisted at the end of the 21 day period and showed no signs of resolving. The balance of evidence across all four of these studies, and taking into account that one (the BASF study) should be given higher weighting due to the fact that the procedure used was fully in line with the guideline and better represents the hazard to humans, clearly indicates that EGBE is an eye irritant but that effects are reversible. A category 2 classification seems most appropriate.

There are a number of supporting studies where data were not reported at the individual animal level so a full comparison with the CLP classification criteria cannot be made. Nevertheless, these studies provide valuable useful additional information on the eye irritancy potential of EGBE (Table 2). In terms of acute effects, they support a classification as category 2. Iritis effects are well below the threshold that would trigger classification as category 1. The corneal effects do seem to be rather variable, and some studies do show a severity that could be deemed sufficient to trigger category 1, but these effects are transient, with a reduction in severity by the 96 hour observation. Most of the studies were only continued for 7 days. For the two studies that extended beyond this, all lesions had disappeared by 14 days, which adds to the weight of evidence that effects are reversible.

There are a number of further studies available on the eye irritation potential of EGBE when diluted. These can provide further supporting information on an appropriate classification, including whether the default specific concentration limits are appropriate. The available studies are shown in table 3. The study by Kennah provides the most information which is both qualitative and quantitative in nature (Draize score in the form of an MMAS and percentage corneal swelling). From this study, it is apparent that EGBE remains irritant down to 30%, with a MMAS score in the range of the results seen with pure EGBE (see table 1) but by 20%, the degree of irritation produced is negligible. (A MMAS score of 1 or 2 could only result from the mildest of transient conjunctival effects). This is supported by the other studies which, in combination, show the following (for full details see table 3):

Concentration	Result	Reference
30%	Irritating (MMAS=39)	Kennah (1989)
20%	Minimally irritating (MMAS=2)	Kennah (1989)
15%	Moderate corneal injury	Dow (1980)
10%	Not irritating (MMAS=1)	Kennah (1989)
10%	Slight conjunctival redness	Hoechst (1966)
5%	No adverse effects	Dow (1980)
1%	No effects	Hoechst (1966)

This data again supports a classification as category 2. The data also indicates that the default specific concentration limit of 10% for classification of mixtures is conservative; a limit of 20% would appear to be justifiable based on the available data.

In vitro studies available for EGBE

The CLH dossier, in the summary on eye irritation, refers to the results from the two HET-CAM assays as supportive of the proposal that EGBE should be classified as causing severe eye damage. In vitro eye irritation studies are normally used for screening purposes. They cannot, certainly in isolation, be used to compare against the classification and labelling criteria for eye irritation and should not be used when in vivo data is also available, particularly in this case when there are a large number of in vivo studies available that, in general, present consistent results. For the hens egg test (HET, alternatively known as the Hühner-embryonen test on chorioallantoic membrane (HET-CAM) or simply the CAM assay), there are two studies available repeated in the same laboratory (BASF, 2004a; 2004b). The results suggest the substance is strongly irritating. However, the test only ran for 3.5 minutes and can only provide information on immediate irritation potential and not on recovery time, which is the crucial issue for this substance. In addition, recent evaluations of the HET-CAM assay have suggested results should be used with caution. Wilson (2015) in a recent overview of current techniques for ocular toxicity testing noted that the large number of variables in the protocol (Incubation times, relative humidity, number of replicates, breed of hen, criteria for egg selection, egg rotation, method of opening the eggshell, volume/weight/concentration of test substance used, use of positive/negative controls and exposure time) leads to problems regarding intra-laboratory reproducibility. Wilson notes that the method has yet to receive international regulatory acceptance. ICCVAM (2010) recommends that the test is used for non-regulatory validation or optimization studies. ICCVAM concludes that the accuracy and reliability of the HET-CAM test method does not support its use as a screening test to distinguish substances not labelled as irritants (EPA Category IV, EU Not Labeled, FHSA Not Labeled) from all other hazard categories (EPA Category I, II, or III; EU R41 or R36; FHSA Irritant) when results are to be used specifically to classify and label substances under the EPA, EU, or FHSA classification systems. Taking this into consideration along with the plethora of in vivo data, we believe that the HET-CAM results should be discounted completely from consideration regarding this end point.

The comment that the test results of EGBE at 10% in this study show strong irritation potential do not have any scientific basis and should be compared to the available in vivo data at dilutions that show negligible effects at 10% (see table 3.) There is other ex vivo data not included in the CLH document (Kennah, 1989) which shows that EGBE has very low irritation potential at dilute concentrations and suggests that the cornea swelling test over-estimates irritation potential.

Other data on the substance.

The CLH dossier includes other data proposed as relevant to this end point. The human volunteer study (Carpenter, 1956) is not relevant for the eye irritation end point as the exposure is to vapours and reports of 'irritation' to the mucous membranes are more likely to be reports of discomfort and annoyance.

The CLH dossier also proposes three further studies in rats as evidence of irritation potential. Two of these are by the oral route at doses known to cause haemolysis and some of the effects observed are secondary to this (Nyska, 1999, 2003). The inhalation teratology study noted periocular wetness which the CLH dossier author suggests could be due to irritation (Tyl (1984).

None of this data provides any useful contribution a decision on this end point.

Studies available for similar substances

We believe it is also instructive to look at other similar substances to gauge the eye irritancy potential. The substance triethylene glycol butyl ether (TEGBE – 2-(2-(2-butoxyethoxy)ethoxy)-ethanol) is a member of the same homologous series as EGBE (also known as monoethylene glycol butyl ether). TEGBE has a substantial amount of eye irritation data available, including on formulations containing different dilutions of the substance. The data shows a similar pattern of response to EGBE, i.e. that the acute lesions are not particularly severe but they are persistent and can last for at least 21 days. There is no recent study on TEGBE that uses washing after 24 hours as there is with EGBE to examine the effect of this on persistence.

TEGBE was reviewed at a European level in 2001 and a harmonised classification for eye irritation agreed. In the absence of data showing that washing could reduce persistence, a classification of R41 was agreed, but based on the data submitted on formulations containing TEGBE, specific concentration limits were agreed that were far less severe than the default[1]. We believe that this data can be used to support the case that the eye irritation potential of the structurally similar substance EGBE declines to the extent that it can be regarded as negligible at concentrations of 20% and below. The industry submission made to support the proposal for TEGBE is included as a separately submitted document with the name "F031 TEGBE Eye Irritation data 30 11 2001.pdf".

The harmonised classification for 2-(2-butoxyethoxy)ethanol, a third member of the ethylene glycol butyl ether homologous series, is Eye Irritant category 2 and the available data indicates that this is correct.

Broader considerations in the interpretation of data from the Draize test

The author of the CLH dossier notes that washing the eyes is permitted in the OECD TG405 study guidelines but expresses the opinion that this has the potential to underestimate the severity of the effects. We believe that the scientific data supports the opposite and that washing will produce a more accurate representation of the situation in humans and that not washing is likely to over-estimate the effects. The eyes of rabbits are generally more susceptible to irritating substances than the eyes of humans (Wilhelmus, 2002). This is due to differences in blink frequency and ocular surface chemistry. Humans also have a much more pronounced lachrymatory response to eye irritation than rabbits and this produces a natural irrigation of the eye. Freeberg (1986) carried out a study to compare the eye irritation effects between rabbits and human volunteer using a number of formulated cleaning products. This study also investigated the clearance time from the eye of the introduced chemical insult. For the Draize dose of 0.1ml, the average clearance time for humans

[1] Harmonised classification is <20% not classified, 20-30% eye irrit 2, >30% eye irrit 1.

across the whole study was 16 hours (SD=12) whereas for rabbits it was 61 hours (SD=20). This suggests that washing at 24 hours is a much better representation of the situation for humans. York (1998) in his review of the Draize test noted that care needs to be taken in the interpretation of the number of days to heal, particularly for minor lesions such as redness and that interpreting such lesions as evidence of 'severe damage' can be counterproductive. York suggested that other factors that might lead to 'days to heal' in rabbits as not being a good indicator of irritancy in humans include persistence under the nictating membrane and secondary infections resulting from the initial insult. The first of these can be addressed by washing and the second could contribute to isolated incidents of persistence of effects at 21 days. Freeberg, York and Wilhelmus all established that the use of smaller volumes for the test (0.01mL) produced a more representative response in rabbits compared to humans. There are no reliable studies that use volumes less than 0.1ml although the studies that use 0.1ml of diluted EGBE can be considered indicative, for instance 0.1ml of a 10% solution of EGBE produced only slight conjunctival redness – more consistent with a category 2 than a category 1 eye irritant.

In a very recent publication, Barrosa et al (2017) reviewed a large database of eye irritation studies. This review was undertaken by a number of experts from the cosmetics industry and other third parties to support validation activities of non-animal test methods. This included a review of the most important end point drivers of classification from in vivo. The authors also carried out a critical review of the GHS classification criteria and came to the following conclusions:

1. Classification based on corneal opacity effects remaining in a single animal after 21 days are questionable grounds for classification as a category 1. The authors stated the following: "*It is questionable whether this type of effects should lead to a Cat 1 classification, especially the cases of delayed effects observed in a single animal as these occur probably for reasons unrelated to the test chemical, such as mechanical abrasion due to prolonged exposure, microbial infection (the so-called secondary inflammatory process), and/or differences in animal behaviour. Many of these studies were probably conducted before the 2002 update of OECD TG 405 when rinsing of the eye was not allowed before the 24-h reading. This may have led to a large variation of contact time between the test chemical and the eye from a couple of minutes to 24 h in different animals and may have thus led in some cases to an exacerbated exposure that could explain the discordant effects observed in a single animal (or the minority of the animals). To add to this, animals are immediately released into their home cages after treatment, where they can move freely. While some animals may immediately start grooming and/or scratching and do this excessively, others may not react at all. These variations in behaviour are another important source of variability between animals.*" It is notable that this is the situation seen with EGBE. Across the three reliable studies that ran for 21 days, corneal opacity was only seen in 1 animal out of 12 at the 21 day observation.
2. Classification base on low level conjunctival effects (level 1) remaining at day 21 is highly questionable. The note that other classification schemes, such as that of the US EPA (1998) consider conjunctival effects (redness and chemosis) of <2 as cleared. Barrosa points out that conjunctival redness scores can exceed 2 in a least one animal over days 1-3 yet lead to no classification, which supports the US EPA position of considering effects less than a score of 2 to be insignificant. The biological relevance of persistence of conjunctival effects in driving Cat 1 classification in the absence of any other Cat 1 triggering effects is also questioned by Adriaens et al. (2014). Overall, the evidence supplied by Barrosa et al lead to them strongly recommending that chemicals should not be classified as Cat 1 based on CR and/or CC scores of 1 at day 21 in the absence of any other cat 1 triggering effects. It is notable that this is the situation with EGBE. Across the three reliable studies that ran for 21 days, conjunctival effects

were seen in only one study on day 21 and these were only to level 1 and no other effects persisted.

Barossa et al concluded, that as a consequence of their analysis, there is a need for a critical revision of the UN GHS/EU CLP decision criteria for the Cat 1 classification of chemicals for eye irritation.

Conclusion

The available data on this substance clearly supports a classification of category 2 for eye irritancy based on the immediate irritation symptoms produced consistently in a large number of in vivo studies. However, the crucial issue in the decision is the recovery time from the effects produced. There are only three studies available that were continued for 21 days sufficient to observe reversibility over the period stipulated in current guidelines (BASF 2000; ECETOC, 1998; Safepharm, 1994)[2]. In each of the latter two studies, one animal had some residual symptoms at the end of the 21 day period, whereas the BASF study showed full reversibility. 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. In both cases the residual lesions seen are questionable as to their relevance as an indicator of irreversible effects or, in the case of isolated findings, their unambiguous link to substance exposure. 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.

The consortium members of the REACH registration of EGBE do not agree with the category 1 classification as proposed in the submitted Annex XV dossier. Based on a considered evaluation of all the available data, a classification for eye irritancy as category 2 seems most appropriate along with a specific concentration limit of 20%.

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[2] Although there are two older/non guideline studies that showed reversibility over 14 days (BASF, 1960; Kennah, 1989)

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Studies where data is available in sufficient detail at individual animal level to enable comparison with CLP criteria (Table 1)

Protocol/GLP [Klimisch rating]	**# animals**	**Washing @ 24hrs**	**Average score of worst 2/4 animals (24, 48, 72hr)**	**Classification conclusion based on effects in first 72 hours**	**MMAS score (out of 100)**	**Duration of observation**	**Effects seen at end of study**	**Reference**
OECD405/Yes [1]	3	Yes	Cornea: 1.0 Iris: 0.7 Redness: 2.7 Chemosis: 2.0	Yes, Cat 2 *conjunctival (redness) effects*	30	21 days	No	BASF (2000)
US EPA 1500.42/No [2]	6	No	Cornea: 1.2 Iris: 0.8 Redness: 2.8 Chemosis: 2.8	Yes, Cat 2 *corneal, chemosis and conjunctival effects*	37	7 days	Yes but only 7 days	Huntingdon (1979)
OECD405/Yes [2]	3	No data but likely no	Cornea: 2.5 Iris: 1.5 Redness: 2.5 Chemosis: 3.0	Yes, Cat 2 *corneal, chemosis and conjunctival effects*	69	21 days	Yes*, chemosis (1 animal), conjunctival effects (2 animals)*	ECETOC (1998)
OECD405/Yes [1]	6	No	Cornea: 1.1 Iris: 1.0 Redness: 2.0 Chemosis: 2.0	Yes, Cat 2 *corneal and conjunctival effects (latter only just)*	39	21 days	Yes, *Circumcorneal vascularisation (1 animal but out of 6), persistence ectropion (some animals)*	Safepharm (1994)

Notes:

Trajectory of key lesions of study reported in ECETOC reference: Redness:

Animal	24hr	48hr	72hr	7 day	21 day
1	**2**	**2**	**2**	**2**	**0**
2	**3**	**3**	**3**	**2**	**1**
3	**2**	**2**	**2**	**1**	**1**

These results show that the lesions were healing and likely would have resolved within a further week.

Trajectory of the key lesions seen in the Safepharm study: Corneal opacity

Animal	24hr	48hr	72hr	7 day	21 day
1	**1**	**1**	**1**	**2**	**2**
2	**1**	**1**	**2**	**1**	**0**
3	**1**	**1**	**1**	**0**	**0**
4	**1**	**1**	**1**	**0**	**0**
5	**1**	**1**	**1**	**1**	
6	**1**	**1**	**1**	**0**	**0**

This show persistence in one animal out of six, which for unexplained reasons showed no signs of resolving.

It is instructive to pool the results from all studies and take the average of the worse 12 of the 18 animals (selected on the basis that the classification criteria is based on the results of the worse of 2 out of 3 or 4 out of 6 animals, ie the worst 66%). This approach reveals the following scores: Corneal effects: 1.4, Iris effects: 1.0, Redness: 2.5, Chemosis: 2.6. These are scores that are consistent with a category 2 classification for eye irritancy.

Studies where data available not in sufficient detail at individual animal level to enable comparison with CLP criteria (Table 2)

Protocol	**# animals**	**Average score (24, 48, 72hr)**	**Classification based on effects in first 72 hours**	**Duration of observation**	**Effects seen at end of study**	**Comment**	**Reference**
OECD405	6	Cornea: 1.3 Iris: 1.0 Redness: 2.6 Chemosis: 0.8	Yes, Cat 2 *corneal, iris and conjunctival effects*	7 days	Yes	Scores reported only as averages	Jacobs (1987)
OECD405	3	Cornea: 2.1 Iris: 1.0 Redness: 2.4 Chemosis: 1.0	Yes, Cat 1 *effects*	7 days	Yes	Scores reported only as averages	Jacobs (1987)
OECD405	6	Cornea: 1.7 Redness: 2.5 Chemosis: 0.9 Corneal swelling: 70%	Yes, Cat 2 *corneal, iris and conjunctival effects*	7 days	Yes	Scores reported only as averages	Jacobs (1988)
OECD405	Not indicated	Cornea: 1.7 Iris: 0.8 Redness: 2.5 Chemosis: 0.8	Yes, Cat 2 *corneal and conjunctival effects*	4 days	Yes	Scores reported only as averages. Corneal effects clearly reducing by 96hr observation (end of study)	Parent (1992)
BASF-(Draize)	2	Cornea: 3.0 Iris: n/a Redness: 2.0 Chemosis: 2.0	Yes, Cat 2 *corneal, chemosis and conjunctival effects* Yes (margin*al*) cat 1 *based corneal effects*	14 day	No	Iris effects not reported. Corneal effects just reached level that could trigger cat 1 classification Scores reported only as averages	BASF (1960)
BASF-(Draize)	2	Cornea: 1.0 Iris: 0.5 Redness: no effects Chemosis: 0.5 (24 hr averages only)	Yes, Cat 2 *corneal effects (based on limited data available)*	8 day	Yes	Supports category 2 classification but rated unreliable as no data available at 48 and 72 hours.	BASF (1968)

Draize and corneal swelling	4-6		Not possible to determine. MMAS = 66. Corneal swelling 181%	14 days	No	Supports category 2 classification	Kennah (1989)
Enucleated rabbit eye test	n/a	n/a	Corneal swelling 130% after 5 hours	5 hours	No	Supports category 2 classification	Jacobs (1987)

Notes:

All studies rated as Klimisch 2. None carried out to GLP. None carried out eye washing at 24 hours.

The two Jacobs and Parent publications are from the same period and may refer to the same experimental work. The results are very similar but not exactly identical and may result from averages across what was at least two separate studies performed by Jacobs (1987). Jacobs (1988) in particular is likely to be a publication of Jacobs (1987) and an average of these two experimental studies.

A pooled average weighted for the number of animals in the studies for the acute effects across the 24, 48 and 72 hour measurements for the two Jacobs (1987) studies and the BASF (1960) study is as follows: Corneal effects 1.8, Iritis 1.0, Redness 2.4, Chemosis 1.1. This approach suggests that a category 2 classification for eye irritation is most appropriate.

Studies on diluted substance (Table 3)

<table>
<tr><th>Protocol/GLP</th><th># animals</th><th>Klimisch rating</th><th>Results at different dilutions</th><th>Comment</th><th>Reference</th></tr>
<tr><td>Draize and corneal swelling</td><td>4-6</td><td>2</td><td>
<table>
<tr><th>Concentration</th><th>Draize score</th><th>% swelling</th><th>Comment</th></tr>
<tr><td>100%</td><td>66</td><td>181</td><td>Severely irritating, resolution with 14 days</td></tr>
<tr><td>70%</td><td>49</td><td>146</td><td>Moderately irritating</td></tr>
<tr><td>30%</td><td>39</td><td>114</td><td>Moderately irritating, mainly conjunctival effects</td></tr>
<tr><td>20%</td><td>2</td><td>113</td><td>Minimally irritating</td></tr>
<tr><td>10%</td><td>1</td><td>91</td><td>Not irritating</td></tr>
</table>
</td><td>Control value for swelling is 100%. Kennah proposed 70%* increase as a threshold for classification as an eye irritant under the criteria of the Dangerous Substances directive 67/548.

*Jacobs in enucleated eye study (see table 2) proposed 60%.</td><td>Kennah (1989)</td></tr>
<tr><td>No data but presumed Draize</td><td>No data</td><td>2</td><td>
<table>
<tr><th>Amount instilled</th><th>Concentration</th><th>Result</th></tr>
<tr><td>0.005ml</td><td>100%</td><td>Severe corneal injuries with iritis</td></tr>
<tr><td>0.5ml</td><td>15%</td><td>Moderate corneal injury</td></tr>
<tr><td>0.5ml</td><td>5%</td><td>No effects seen</td></tr>
</table>
</td><td>Only basic details available. Lower concentrations used 5x normal instillation volume.</td><td>Dow (1980)</td></tr>
<tr><td>No data but presumed Draize</td><td>No data</td><td>4</td><td>
<table>
<tr><th>Concentration</th><th>Result</th></tr>
<tr><td>100%</td><td>Severe redness and chemosis, corneal opacification</td></tr>
<tr><td>10%</td><td>Slight conjunctival redness</td></tr>
<tr><td>1%</td><td>No effects</td></tr>
</table>
</td><td>Original report not available. Cited in EU risk assessment.</td><td>Hoechst (1966)</td></tr>
<tr><td>No data but presumed Draize</td><td>No data</td><td>4</td><td>Only 5% solution tested. No adverse effects seen</td><td>Minimal experimental detail reported</td><td>Bagley (1994)</td></tr>
</table>

Notes: None of the studies were performed to GLP