Committee for Risk Assessment

RAC

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

14 September 2018

CLH-O-0000001412-86-226/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **2-butoxyethanol; ethylene glycol monobutyl ether**

EC Number: **203-905-0**

CAS Number: **111-76-2**

The proposal was submitted by **Germany** and received by RAC on **1 August 2017.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **17 October 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **1 December 2017**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Bogusław Barański**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **14 September 2018** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	603-014-00-0	2-butoxyethanol; ethylene glycol monobutyl ether; butyl cellosolve	203-905-0	111-76-2	Acute Tox. 4* Acute Tox. 4* Acute Tox. 4* Skin Irrit. 2 Eye Irrit. 2	H332 H312 H302 H315 H319	GHS07 Wng	H302 H312 H332 H315 H319			
Dossier submitter's proposal	603-014-00-0	2-butoxyethanol; ethylene glycol monobutyl ether	203-905-0	111-76-2	**Retain** Skin Irrit. 2 **Add** STOT RE 2 **Modify** Acute Tox. 3 Acute Tox. 3 Acute Tox. 4 Eye Dam. 1	**Retain** H315 **Add** H373 (blood) **Modify** H331 H311 H318	**Add** GHS05 GHS06 GHS08 Dgr **Remove** GHS07 Wng	**Retain** H302 H315 **Add** H373 (blood) **Modify** H331 H311 H318		**Add** inhalation: ATE = 3 mg/L dermal: ATE = 300 mg/kg bw oral: ATE = 500 mg/kg bw	
RAC opinion	603-014-00-0	2-butoxyethanol; ethylene glycol monobutyl ether	203-905-0	111-76-2	**Retain** Acute Tox. 4 Skin Irrit. 2 Eye Irrit. 2 **Remove** Acute Tox. 4* **Modify** Acute Tox. 3	**Retain** H302 H315 H319 **Remove** H312 **Modify** H331	**Add** GHS06 Dgr **Remove** GHS07 Wng	**Retain** H302 H315 H319 **Modify** H331		**Add** inhalation: ATE = 3 mg/L oral: ATE = 1200 mg/kg bw	
Resulting Annex VI entry if agreed by COM	603-014-00-0	2-butoxyethanol; ethylene glycol monobutyl ether	203-905-0	111-76-2	Acute Tox. 3 Acute Tox. 4 Skin Irrit. 2 Eye Irrit. 2	H331 H302 H315 H319	GHS06 Dgr	H331 H302 H315 H319		inhalation: ATE = 3 mg/L oral: ATE = 1200 mg/kg bw	

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity: oral route

The Dossier submitter (DS) provided results of 16 acute oral toxicity studies: 9 studies in rats, 4 studies in mice, 1 study in rabbits and 2 studies in Guinea pigs (see Table 9 of the Background document).

Rats

The oral LD_{50} values in rats were in the range of 470 – 2000 mg/kg bw, except for 1 study in which they were reported to be in the range of 560 – 2800 mg/kg bw for males and 530 - 2300 mg/kg bw for females rats.

Mice

The oral LD_{50} values in mice were in the range of 100 – 1519 mg/kg bw, except for 1 study in which they were reported to be 2005 mg/kg bw for fed mice, but 1519 mg/kg bw for fasted mice.

Rabbits

The oral LD_{50} values in rabbits were in the range of 320 – 370 mg/kg bw.

Guinea Pigs

The oral LD_{50} values in in Guinea pigs were in the range of 1414 – 1200 mg/kg bw.

The DS also presented human data on acute oral toxicity (see Table 10 of the Background document) based on observations made after suicide attempts or accidental poisonings with 2-buthoxyethanol. Doses of 2-buthoxyethanol causing severe poisoning with coma, breathing difficulties, acidosis, hypoxemia, anemia, haematuria were estimated in 5 cases to be in the range of 0.5 – 1.5 g/kg bw, and in 1 case about 4.5 g/kg bw.

Based on results of the animal studies, the DS proposed to classify 2-buthoxyethanol in Category 4 for acute oral toxicity (Acute Tox. 4; H302, Harmful if swallowed), since most LD_{50} values were in a range of 300 – 2000 mg/kg bw, with an ATE value of 500 mg/kg bw.

Acute toxicity: dermal route

The DS provided results of 15 acute dermal toxicity studies: 3 studies in rats, 8 studies in rabbits and 4 studies in Guinea pigs (see Table 12 of the Background document).

Rats

The dermal LD_{50} values in rats were above 2000 mg/kg bw.

Guinea Pigs

The dermal LD_{50} value in Guinea pigs in an OECD TG 402, GLP study (Eastman-Kodak, 1994a) was above 2000 mg/kg bw. In 2 non-guideline studies (Roudabush *et al*., 1952; Wahlberg and

Boman, 1979) the dermal LD_{50} values were in the range of 230 – ≤ 1800 mg/kg bw, and in 1 non-guideline study (Mellon Institute, 1952) it was 6411 mg/kg bw.

Rabbits

The dermal LD_{50} values in rabbits, in 6 studies similar to OECD TG 402 with occlusive application, were respectively: 560 mg/kg bw, 680 mg/kg bw, 580 mg/kg bw, 100 mg/kg bw, 569 mg/kg bw, 435 mg/kg bw. In addition, 2 GLP studies, similar to OECD TG 402, were available; 1 had an LD_{50} value above 2000 mg/kg bw (semi-occlusive application) and 1 had a dermal LD_{50} value of 841 mg/kg bw (occlusive dermal application).

Humans

No human data on acute dermal toxicity was reported.

Based on results of the animal studies the DS proposed to classify 2-buthoxyethanol in Category 3 for acute dermal toxicity (Acute Tox. 3; H311, Toxic in contact with skin), since most dermal LD_{50} values for rabbits were in a range of 200–1000 mg/kg bw, with a proposed ATE value of 300 mg/kg bw.

Acute toxicity: Inhalation route

The DS provided results of the acute inhalation toxicity studies: 5 studies in rats, 1 study in mice and 2 studies in Guinea pigs (see Table 13 of the Background document).

Rats

The LC_{50} values in rats were in the range of 2.2 - 4.92 mg/L/4h, except in 1 study in which an LC_{50} of 12.36 mg/L/4h was calculated (Gage, 1970). One study was aimed at measuring 0-lethality time for which at least 1 death was found, and no LC_{50} could be established (Klimisch *et al*. 1988).

Mice

The LC_{50} values in mice was 4.12 mg/L/4h.

Guinea Pigs

The LC_{50} values in Guinea pigs was 7.65 mg/L/4h in 1 study, and no mortality was observed in second study using a lower concentration of 633-691 ppm for 1 hour whole body exposure.

Human data

In studies on human volunteers a 4-h exposure to 2-buthoxyethanol at concentration of 0.48, 0.55 or 0.95 mg/L induced irritation to the eyes, nose and throat, a disturbance of taste, a slight increase in nasal mucous discharge and headache; women appeared to be more sensitive to the induction of these effects than men. There was no evidence of changes from pre-exposure values in erythrocyte fragility, blood pressure, pulse rate or urinary levels of glucose or albumin. Concerning haematology, there were no adverse effects seen at either exposure concentration (Carpenter *et al*., 1956).
No consistent effect on the lungs or the heart and no overt signs of toxicity were observed in 2 studies with male volunteers exposed to 2-buthoxyethanol for 2 hours at concentration of 0.24 mg/L (Johanson, 1986; Johanson and Bowman, 1991).

Based on results of the animal studies, the DS proposed to classify 2-buthoxyethanol in Category 3 for acute inhalation toxicity (Acute Tox. 3; H331, Toxic if inhaled), since the LC_{50} values of 2-

buthoxyethanol vapour for rats, mice and Guinea pigs were in a range of 2–10 mg/L, with a proposed ATE value of 3 mg/L.

Comments received during public consultation

One MSCA supported classification for acute oral, dermal and inhalation toxicity, but disagreed with the proposed ATE values by all routes, noting that LD_{50} values based on the existing database should be used as ATE, instead of the standard values in Annex I, Table 3.1.2 of the CLP Regulation.

In response, the DS agreed and proposed that the lowest oral LD_{50} value in rabbits (320 mg/kg bw), the lowest dermal LD_{50} (435 mg/kg bw) in rabbits and the lowest LC_{50} in rats (2.2 mg/L/4h) are appropriate ATE values for 2-butoxyethanol while classifying mixtures containing that substance.

One industry or trade association and one company-manufacturer, agreed with the proposed classification for acute oral toxicity, but disagreed with the proposed modification of the current classification for acute dermal and inhalation toxicity. For acute dermal toxicity, it was argued that clinical and pathological findings in rabbits can be secondary to the haemolysis caused in rabbits by this substance. Therefore the studies with this species, as they are considered to be less sensitive than humans for this specific effect, should be excluded when classifying for acute dermal toxicity. The acute dermal toxicity studies with Guinea pigs, which are considered to be resistant to haemolysis caused by 2-buthoxyethanol, should be used instead as they are a better model for human toxicity. For acute inhalation toxicity, they noted that due to low volatility and low vapour pressure of 2-buthoxyethanol (≈80Pa at 20°C) there is no human acute toxicity hazard from inhalation exposure to 2-butoxyethanol and that classification via this route is not warranted.

One company-manufacturer agreed with the proposed classification of 2-buthoxyethanol for the oral route (Acute Tox. 4; H302), but not the proposed classification via the dermal and inhalation route. The company emphasised that rat, mouse, rabbit, hamster and baboon are sensitive to haemolysis induced by butoxy acetic acid (BAA, the primary metabolite of 2-butoxyethanol), whereas human, Guinea pig, dog and cat are resistant to BAA-induced haemolysis. Due to the similarity in sensitivity between humans and Guinea pigs, they suggested to use the LD_{50} values for Guinea pig when assessing classification for acute toxicity. Classification based on the LD_{50} values for rat, mouse and rabbit would lead to an overestimation of the hazard.

The DS did not agree with the interpretation by the industrial organisations, noting that the rabbit is to be considered the most sensitive species for acute dermal toxicity and that results of acute dermal toxicity studies in rabbits (7 out of 8 performed) are consistently demonstrating low LD_{50} values. In the opinion of the DS, the assumptions that the cause of death in rabbits might be solely due to haemolysis, and that BAA is the only metabolite responsible for the haemolytic effects in rabbits, are not sufficient to exclude studies on rabbits. This is particularly 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 that *"in general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested"*. The DS further noted, that 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 (with uncertainties on the dose ingested) in the region of 400 mg/kg bw, while the rabbit LD_{50} was 320 mg/kg. Moreover, it has to be considered that interindividual variation can be very high in humans. Moreover, high interindividual variation in permeation, absorption and elimination of 2-butoxyethanol was demonstrated in studies performed on human volunteers. This indicates the 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.

The DS also disagreed with the comments that classification for acute inhalation toxicity is not justified. Low vapour pressure is not a sufficient argument since assumption of potential low exposure is related to risk assessment and not to hazard assessment. The DS also noted that a value of 4.4 mg/L, as proposed by the consortium as the maximal sustainable vapour concentration, is quite uncertain and not compatible with experimental data. For example, in a study by Tyler (1984), a higher concentration (6.4 mg/L/7h) was used and 50% mortality was observed in Guinea pigs at that concentration. The DS further remarked that there seem to be further acute inhalation toxicity studies, which were not cited in the CLH report, but which were submitted during public consultation. Two studies in rats (BASF 1968 and 1978) indicated an LC_{50} between 1.1 and 5.3 mg/L/4h, 1 study in dogs (Dow, 1974) indicated an LC_{50} > 2.36 mg/L/4h), 1 in rabbits (Dow, 1974) indicated an LC_{50} ~ 2.36 mg/L/4h, and 1 in Guinea pigs (Dow, 1974) showed an LC_{50} > 2.36 mg/L/4h. The DS concluded, that taking into account these additional data, classification for acute inhalation toxicity in Category 3 is still to be considered justified (CLP criteria for vapour, Cat. 3: 2 > ATE ≤ 10 mg/L/4h).

Assessment and comparison with the classification criteria

The main mechanism of systemic toxicity of 2-buthoxyethanol is haemolysis of erythrocytes caused by its metabolite butoxy acetic acid (BAA) (EU RAR, 2006). There are considerable interspecies differences in sensitivity to this toxic action between animal species and humans. As reported in the EU RAR (2006), Guinea pigs and humans are relatively resistant, rodents are very sensitive (rats are 30 times more sensitive than humans), while rabbits are less sensitive than rodents, but more sensitive than humans and Guinea pigs.

Acute human toxicity data comes from accidental ingestion by children or suicide attempts by adults with mixtures containing 2-buthoxyethanol. A number of human case studies suggest that the human LOAEL after ingestion of 2-buthoxyethanol, with the main toxic effect being metabolic acidosis and sometimes haematotoxicity, is in the region of 400 mg/kg bw. No human deaths were reported after the estimated ingested doses of 0.5 – 4.5 g/kg bw (EU RAR, 2006).

Therefore, to ensure relevance for human hazard assessment, RAC is of the opinion that the lowest oral LD_{50} of 1200 mg/kg bw for Guinea pigs, a species reportedly having similar sensitivity as humans to the haemolytic effect of 2-buthoxyethanol, should be chosen as the oral ATE value.

Acute toxicity: oral route

Taking into account that the oral LD_{50} values of 2-buthoxyethanol in several acute oral toxicity studies, in rats (from 620 up to 1950 mg/kg bw), in mice (1230 – 1519 mg/kg bw), in rabbits (320 – 370 mg/kg bw) and in Guinea pigs (1200 – 1414), were within the classification criteria of 300 – 2000 mg/kg bw for Category 4, RAC is of the opinion that 2-butoxyethanol **warrants classification as Acute Tox. 4; H302 (Harmful if swallowed). The ATE for classifying mixtures should be equal to the lowest oral LD_{50} for Guinea pigs, that is 1200 mg/kg bw.**

RAC noted that from the acute human toxicity data, the range of doses which lead to clinical symptoms varies between 0.5 and 4.5 g/kg bw. In all cases, patients exhibited CNS depression (coma) and metabolic acidosis. Signs of haemolysis were seen in some cases but this finding was not systematic. The data shows that in humans CNS depression (coma) and metabolic acidosis might be the main symptoms of acute intoxication since humans are more resistant to the acute haemolytic effects of 2-butoxyethanol than rodents.

Acute toxicity: dermal route

The dermal LD_{50} values for 2-buthoxyethanol in several acute dermal toxicity studies in rats were above 2000 mg/kg bw, in rabbits in the range of 100 mg/kg bw – > 2000 mg/kg bw, and in Guinea pigs 230 – 6411 mg/kg bw. However, the lowest dermal LD_{50} for Guinea pigs comes from a study by Roudabush *et al*. (1965), not in compliance with current test guidelines and not according to GLP, and differs considerably from LD_{50} values for Guinea pigs of > 2000 mg/kg bw obtained in more recent studies. The lowest dermal LD_{50} for Guinea pigs of 230 mg/kg bw is also 5 times lower than available oral LD_{50} for Guinea pigs (1200 – 1414 mg/kg bw), and can be noted that the absorption of 2-butoxyethanol by the oral route in rats and mice is relatively high (assumed to be 100%) as reported in section 8.1 of the background document, while dermal absorption is less effective, amounting to 20 and 30% of the administrated dose. Therefore, the study of Roudabush *et al*. (1965) is not considered reliable and its results should not be used for classification. Since rabbits are reportedly more sensitive than humans to the acute toxicity of 2-butoxyethanol, the LD_{50} in this species is less relevant for classification compared to Guinea pig data. Taking into account data from studies in Guinea pigs and rats, showing dermal LD_{50} above 2000 mg/kg bw, RAC is of the opinion that **2-butoxyethanol does not warrant classification for acute dermal toxicity.**

Acute toxicity: Inhalation

The LC_{50} values of 2-buthoxyethanol in several acute inhalation toxicity studies in rats were in the range of 2.21 – 4.92 mg/L/4h, in 1 study in mice 4.12 mg/L and in 1 study in Guinea pigs 7.65 mg/L/4h; thus, they were all within the classification criteria of 2 – 10 mg/L for Acute Tox. 3. It is noted that due to low volatility and low vapour pressure of 2-buthoxy- ethanol the Guinea pigs could have been exposed not to pure vapour but to a mixture of vapour and mist of 2-buthoxyethanol, since the saturated vapour concentration at 20°C is 4.4 mg/L. Hence, the data on Guinea pigs alone are borderline between classification and no classification for acute inhalation toxicity. However, due to this situation RAC took all available studies in rats, mice and Guinea pigs into account, and is of the opinion that 2-butoxyethanol **warrants classification as Acute Tox. 3; H331 (Toxic if inhaled), with an ATE of 3.0 mg/L** (Table 3.1.2 of Regulation (EC) No 1272/2008).

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS provided results of 10 skin corrosion/irritation animal studies: 8 studies in rabbits and 2 studies in Guinea pigs (see Table 15 of the Background document); 3 publications with human data (see Table 16 of the Background document), and 3 acute dermal toxicity studies relevant for skin corrosion/irritation (1 study in rats and 2 studies in rabbits; see Table 17 of the Background document).

Animal studies

There is 1 *in vivo* skin irritation/corrosion study for 2-butoxyethanol in rabbits available, performed according to OECD TG 404 (OECD, 1981), not compliant with GLP (Jacobs and Martens, 1985; Jacobs *et al.*, 1987). Thus, the study was considered relevant and reliable with restrictions. 2-butoxyethanol caused an erythema score of 1.7 (mean of 24 to 72h in all 5 animals), an oedema score of 0.13 (mean of 24 to 72h in all 5 animals) and a maximum degree of eschar formation of 2.0 for all 5 animals and both readings. Individual scores for each animal were not reported. Effects were, furthermore, persistent and not fully reversed at the end of the 14-d

observation period. No effects were noted in control animals. The DS concluded that the results of this study indicate that 2-butoxyethanol is a mild to moderate skin irritant.

In other available studies in rabbits (Grote, 1979a; BASF AG), animals were occlusively exposed to 2-butoxyethanol for a longer period (20-24h) than the 4h exposure period recommended in current, validated OECD TG 404; or no individual or mean erythema/oedema scores were reported for any observation time point (BASF AG, 1960; Zissu, 1995). In addition, information on the reversibility of effects is missing. Therefore, due to lack of the above details, the DS concluded that results from these studies can only be used as the supportive data.
A number of further *in vivo* skin irritation/corrosion studies were reported for 2-butoxyethanol in rabbits and Guinea pigs. However, many of these studies were of unknown reliability (see Table 15 of the Background document), and thus were not considered to provide sufficient information for quantitative interpretation and hence could not be used for classification (Bushy Run Research Center, 1989; Duprat and Gradiski, 1979; Eastman Kodak, 1981a; Eastman Kodak, 1981b; Rohm and Haas Co., 1989; Unilever Research, 1989).

Human data

Three studies in humans were reported, either evaluating the skin irritating effects of 2-butoxyethanol by employing the human (repeated) patch test (Greenspan *et al*., 1995), or indirectly as a side effect in absorption and toxicokinetic studies (Jakasa *et al*., 2004; Johanson *et al*., 1988). In these studies, no skin irritating effects of 2-butoxyethanol were detected, but the skin appeared more wrinkled and less elastic after occlusive and immersive exposure, respectively. After immersion (Johanson *et al*., 1988), the volume of fingers and skin thickness decreased significantly, but effects were fully reversible within 1 day. In the human repeated patch test, 3/203 volunteers exhibited slight erythema and 1/203 volunteers showed definite erythema after the first occlusive exposure (Greenspan *et al*., 1995).

In the reliable *in vivo* assays performed according to OECD TG 404 (OECD, 1981) (Jacobs and Martens, 1985; Jacobs *et al*., 1987) and CFR title 16, section 1500.41 (Grote, 1979a), respectively, mean erythema/oedema scores were below 2.3 and thus, according to the CLP Regulation (Annex I, Table 3.2.2), the DS concluded that the substance shall be classified as not irritating to the skin based on these parameters. However, in both studies, as well as in supportive data, effects were persistent and not fully reversible by the end of the observation period (14 d and 72 h). Furthermore, a pronounced variability of response among animals was reported by Jacobs and Martens (1985) and Jacobs *et al*. (1987), with positive effects directly related to chemical exposure in several, but not all, tested individual animals. Based on these results, the DS concluded that the criteria for skin irritation Category 2 are fulfilled.

Although the CLP Regulation does not contain clear criteria for classification for skin irritation based on human data, data obtained e.g. in the repeated patch test (Greenspan *et al.*, 1995) (Table 16 of CLH report) supports the classification based on animal studies (Skin Irrit., Cat. 2).

The DS proposed to retain the current classification of 2-butoxyethanol as Skin Irrit. 2; H315 (Causes skin irritation).

Comments received during public consultation

Two MSCAs supported retention of the current classification for skin corrosion/irritation (Skin Irrit. 2; H315).

Assessment and comparison with the classification criteria

In the key *in vivo* study performed according to OECD TG 404 (OECD, 1981) (Jacobs and Martens, 1985; Jacobs *et al*., 1987) and in the supportive study (Grote, 1979a), mean erythema/oedema scores obtained for 2-butoxyethanol were below 2.3 in all tested animals. However taking into account persistency of effects, not fully reversed at the end of the 14-d observation period (Jacobs and Martens, 1985; Jacobs *et al.*, 1987; BASF AG, 1960; the latter not according to validated test guideline or GLP), or within 72h (Grote, 1979a), the criteria for skin irritation are fulfilled.

In all studies where the persisted inflammation was observed, no destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, were noted in tested animals. Thus, according to the CLP Regulation (Section 3.2.1.1, Table 3.2.1), 2-butoxyethanol should not be classified as corrosive (category 1).

Considering all available evidence, **RAC concludes that 2-butoxyethanol should be classified as Skin Irrit. 2; H315 (Causes skin irritation).**

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS provided results of 19 eye damage/irritation studies in rabbits and 3 *in vitro* studies in fresh fertilised hen eggs, 1 repeat dose inhalation study in humans and 1 inhalation study in rats and rabbits, as well as 2 publications considered relevant for serious eye damage/eye irritation (Tables 18, 19 and 20 of the background document, respectively).

Animal studies

There are 7 *in vivo* studies on eye irritation/corrosion in rabbits, according to OECD TG 405 or US-FHSA (CFR) protocols, considered relevant and reliable (3 reliable without restrictions and 4 reliable with restrictions), providing sufficient data for classification.

In the study by BASF (2000) (OECD TG 405 and GLP-compliant) 2-butoxyethanol caused moderate damage to the treated eyes; the mean score of cornea opacity for all 3 animals was 1.0, 0.67 and 1.0, respectively, with 2/3 animals showing a mean score ≥ 1.0 (at 24 – 72h). The conjunctivae scores for all 3 were 2.3, 3.0 and 2.3, respectively, and all animals (3/3) exhibited a mean score ≥ 2 (at 24 – 72h). The chemosis score for all 3 animals was 2.0, 2.0 and 1.3, while iritis was only mild with a mean iris score of the 3 animals of 0.33, 1.0 and 0.33, respectively (at 24 – 72h). Two out of 3 animals showed a mean chemosis score ≥ 2 (24 – 72h). All observed effects were reversible within the observation time of the study (21 days). However, the eyes of the rabbits were washed after 24h of exposure. This method is in accordance with OECD TG 405 ("*At 24 hours a washout may be used if considered appropriate.*"), especially with respect to animal welfare. However, after removing the test substance by washing, it cannot affect the eyes during the remaining test period; thus leading to attenuated and potentially underestimated effects of the test substance. This should hence be accounted for in the evaluation of effects.

Five of the 6 other reliable *in vivo* studies were also performed according to OECD TG 405 (3 of the 5 studies were also GLP-compliant; see Table 18 of the Background document). In 3 of the 5 studies, the observation period was, however, only 4 or 7 days and thus no information on reversibility of effects at day 21 after instillation were reported. In the 2 other studies, the observation period was until day 21 after instillation, as recommended by the current, validated OECD TG 405. One further study was conducted according to the CFR test for eye irritants. Here,

the observation period was again only 7 days, and thus no information on reversibility of effects at day 21 after instillation were reported. In all 6 studies, animals were instilled with 0.1 mL undiluted test substance in one eye, while the other served as control. Eyes were not washed during the test period and observations were made at 24, 48, and 72h after instillation and at (several) further time points.

Parent (1992) calculated a mean cornea opacity score of 1.73 (24/48/72h), and a mean conjunctivae score of 2.4 (24/48/72h), but did not report the individual data. Nevertheless, based on the mean scores it can be estimated that in at least 4/6 animals a mean score for cornea opacity of ≥ 1.0 (but < 3.0; mean of 24/48/72h) and in 4/6 animals a mean conjunctivae score of ≥ 2.0 (24/48/72h) was obtained. Due to the shortened observation period (4 days), no data on reversibility of effects were available.

Jacobs and Martens (1987) performed 2 tests with 3 animals each and received very similar results: the mean conjunctivae score of all animals (24/48/72h) were 2.54 in the first and 2.51 in the second test run. Individual scores were again not reported. Based on those scores, it can be assumed that in both experiments, a mean conjunctivae score of ≥ 2.0 was obtained in 2/3 animals. The mean iris scores (24/48/72h) were 1.0 in the first and 1.73 in the second test run, again indicating that in the second experiment 2/3 animals scored ≥ 1.0. A cornea opacity score was only reported for the first test run, however the score of 1.59 (24/48/72h) gives reason to assume an individual score of ≥ 1 (and < 3) in 2/3 animals. Due to the shortened observation period (7 days), no data on reversibility of effects were available.

ECETOC (1998) and Safepharm laboratories (1994b) both reported individual scores for their tests and also observed animals for 21 days. In the experiments reported by ECETOC (1998), 3/3 animals scored ≥ 2.0 for chemosis and for conjunctivae (24/48/72h). Furthermore, 3/3 animals received an iris score, as well as a cornea opacity score ≥ 1.0 (24/48/72h). One animal scored > 1.5 for iritis and > 3 for cornea opacity (24/48/72h). Chemosis effects (1/3 animals) and conjunctivae effects (2/3 animals) were not fully reversible within 21 days. Similarly, 5/6 and 6/6 animals, respectively, scored ≥ 2.0 for redness (conjunctivae score) and swelling (chemosis). Moreover, 5/6 animals had an iris score ≥ 1 (and < 1.5) and for 6/6 animals a cornea opacity score ≥ 1 (and < 3) was obtained. One animal showed signs of distress and had to be sacrificed at day 14. Another animal still showed a cornea opacity score of 2.0 at day 21.

Grote (1979b) performed an eye irritation/corrosion study in 6 rabbits according to an US-FHSA protocol (non-GLP), similar to the OECD TG 405, but the observation duration was only 7 days and the Draize scoring was applied. They obtained a chemosis and conjunctivae score ≥ 2 for all (6/6; 24/48/72h) animals and a cornea opacity score ≥ 1 (and < 3) in 4/6 animals (24/48/72h), whereas the cornea opacity scores of the other 2 animals could not be assessed due to dulling of cornea at certain time points. Due to the shortened observation period (7 days), no data on reversibility of effects were available.

There are also 3 *in vitro* and 1 *ex vivo* study available (Anonymous, 2004a; Anonymous, 2004b; Jacobs and Martens, 1987; Kalweit *et al*., 1990; see Table 18 of the background document). Two of them were performed according to an accepted and validated test guideline and provided sufficient data for a comparison with the classification criteria. They both followed the ICCVAM-recommended HET-CAM protocol, were GLP-compliant and considered relevant and reliable with restrictions. Positive controls were valid, but no information on vascular lysis was given. Due to the individual measurements reported, data could be converted into the appropriate irritant score (IS) method. Both tests gave a positive result (IS > 9) for 100% and 10% 2-butoxyethanol, indicating strong irritancy.

Nine additional *in vivo* eye irritation/corrosion studies for 2-butoxyethanol in rabbits were available, but were not considered reliable or could not be assigned a reliability score (see Table 18 of the Background document).

Human data

Three volunteers were exposed to 100 and 200 ppm of 2-butoxyethanol via inhalation for periods of 2 or 4 hours, separated by a 2-h period of non-exposure (Carpenter *et al.*, 1956). Immediate irritation of the nose and throat, followed by ocular irritation and disturbed taste were reported by all 3 subjects, potentially due to direct contact with 2-butoxyethanol vapour. Whether such 'irritation' was physiological or merely discomfort is not clear.

Conclusion

In 2 reliable *in vivo* studies, some relevant effects were seen which were not reversible within 21 days (chemosis and redness: ECETOC, 1998; cornea opacity: Safepharm, 1994b). Thus, the DS concluded that based on these data, one criterion for serious eye damage ("*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*") was fulfilled, and therefore classification as Eye Dam. 1; H318 (Causes serious eye damage) was proposed.

Comments received during public consultation

One MSCA supported classification of 2-butoxyethanol as Eye Dam. 1; H318.

An industry/trade association organisation and a company-manufacturer did not agree with the proposed change from category 2 to 1 for serious eye damage/irritation classification, explaining that the scientific evidence shows that the proposal is incorrect as regards the hazard to humans.

Assessment and comparison with the classification criteria

According to Table 3.3.1 of the CLP Regulation the classification criteria for irreversible eye effects are as follows:

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 out of 3 tested animals, a positive response of: corneal opacity $\geqslant$ 3 and/or iritis > 1.5 (calculated as the mean score following grading at 24, 48, 72 hours after installation of the test material).

According to Table 3.3.2 of the CLP Regulation the classification criteria for reversible eye effects are as follows:

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 out 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.

There are only 3 *in vivo* studies on eye irritation/corrosion in rabbits, performed according to OECD TG 405 protocol, GLP-compliant, considered relevant and reliable and providing sufficient

data for a comparison with the classification criteria, including an observation period of reversibility of 21 days.

In the study conducted by BASF (2000), considered as key study, all observed effects were reversible within the observation time of the study (21 days). However, in this study eyes of the rabbits were washed immediately before first reading (24h after exposure). According to OECD TG 405: "*The eyes of the test animals should not be washed for at least 24 hours following instillation of the test substance, except for solids, and in case of immediate corrosive or irritating effects. At 24 hours a washout may be used if considered appropriate*". Taking into account that 2-butoxyethanol is a liquid, washing of the eyes 4 hours after instillation fulfils the requirements of OECD TG 405 (OECD, 2017).

In the study 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) 1 animal (1/6) showed a cornea opacity score of 2.0 (vascularisation) at day 21. Moreover, ectropion was seen in some animals from 72h (not reversible in 1 animal at 21 d; 1 rabbit showed signs of distress and had to be sacrificed at day 14). Therefore, the results of these 2 studies fulfil the CLP criteria for classification of 2-butoxyethanol for Eye Damage Category 1.

In other *in vivo* studies in rabbits, performed according to OECD TG 405 or US-FHSA (CFR) protocols, in which the observation period was shorter than 21 days, the following information on reversibility of ocular lesions can be summarised:

- study by Grote, 1979b (US-FHSA CFR, non-GLP): observation duration was only 7 days; cornea opacity, iris, conjunctival redness and conjunctival oedema (chemosis) effects were not fully reversible by the end of the observation period;
- 2 studies by Jacobs and Martens, 1987, Parent 1992 (OECD TG 405, GLP-not specified): observation duration was only 7 days and no data on reversibility of effects were available;
- study by Jacobs, (OECD TG 405, GLP-compliant): observation duration was only 4 days; cornea opacity score of 1.2, iris score of 0.2, conjunctival redness score of 0.2 and conjunctival oedema (chemosis) score of 1.6 at the end of the observation period (96h).

In the *in vivo* study (Jacobs and Martens, 1987), performed according to OECD TG 405 protocol, but with an observation period of only 7 days and no individual scores reported, the mean iris scores (24/48/72h) was 1.73 in all 3 tested rabbits; thus it can be assumed that a mean iris score of ≥ 1.5 was obtained in 2/3 animals. Therefore, the results of this study meet the classification criteria for Eye Damage Category 1.

Two *in vitro* studies (Anonymous, 2004a; Anonymous, 2004b) with 2-butoxyethanol were conducted according to ICCVAM-recommended test method protocol (Hen's Egg Test – Chorioallantoic Membrane; HET-CAM). This test is not currently validated for classification of ocular irritancy and is recommended for use as part of a tiered-testing strategy for regulatory classification and labelling (e.g. Top-Down Approach[1]). The potential ocular irritancy of the test substance is measured by its ability to induce toxicity in the chicken chorioallantoic membrane. The times (in seconds) of appearance of the following effects: (1) haemorrage, (2) coagulation and (3) vessel lysis are noted during 300 seconds after instillation of 0.3 mL of the test substance on the chorioallantoic membrane of chicken embryo, noting particularly findings observed 0.5, 2

[1] The top-down approach should be used when available information suggests that the substance may cause serious eye damage. The bottom-up approach, on the other hand, should be followed only when available information suggests that the substance may not be irritant to the eye.

and 5 minutes after instillation. In these 2 HET-CAM tests, 2-butoxyethanol gave positive results, i.e. IS > 9, indicating that 2-butoxyethanol is causing severe irritation (haemorrhage and coagulation), but lack of data on occurrence of lysis within the chorioallantoic membrane in both tests and unclear scoring system does not make it possible to differentiate between irritation and corrosion.

RAC noted that the *in vivo* studies in rabbits have already been evaluated in the EU RAR (2006) with a conclusion to classify 2-butoxyethanol as Eye Irrit. 2. No new *in vivo* or *in vitro* data with a significant impact on classification were presented in the CLH proposal.

In the key study in rabbits (BASF, 2000), the effects on eyes were fully reversible within an observation period of 21 days; thus these results support classification as Eye Irrit. 2; H319 (Causes serious eye irritation). In this study eyes were washed 24h after instillation, which is in accordance with OECD TG 405. Rinsing the treated eye simulate the human situation more closely, where lacrimation would clear the substance from the eye. In none of the evaluated studies the severity of the eye effects met the criteria for category Eye Dam. 1. In the 2 studies (ECETOC, 1998; Safepharm laboratories, 1994b), performed according to OECD TG 405 protocol and GLP-compliant, in which eyes were not washed, the effects on eyes were not fully reversed within an observation period of 21 days; however the severity of these effects was low, and their reversibility after a longer time period cannot be excluded.

Taking into account all evaluated data, **RAC is of the opinion that classification of 2-butoxy-ethanol as Eye Irrit. 2; H319 (Causes serious eye irritation)**, i.e. the current harmonised classification, **is warranted.**

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS provided results of 27 oral repeat dose toxicity studies with different study durations: 20 studies in rats and 7 studies in mice (see Table 21 of the Background document). Out of the 20 submitted studies, 1 study was performed according to OECD TG 414 (Prenatal developmental toxicity study; Sleet *et al*. 1991) and 1 study according to OECD TG 408; both considered reliable without restriction. Eighteen oral repeated toxicity studies did not follow a specific test guideline, were not GLP-compliant, and considered reliable with restriction.

For inhalation exposure the DS provided the results of 19 inhalation repeat dose toxicity studies with different study durations: 12 studies in rats, 3 studies in mice, 1 study in rabbits, 1 study in Guinea pigs, 1 study in dogs and 1 study in monkeys. The studies were considered reliable with restriction, or not assignable a reliability score.

The DS also provided results of 2 dermal repeat dose toxicity studies in rabbits, and of 2 repeat dose toxicity studies in rats using subcutaneous injections, but their results were not considered appropriate for comparison with classification criteria.

The DS reported that most of the relevant and reliable studies indicated that 2-butoxyethanol causes severe haemolytic anaemia in various species (e.g. rats, mice, rabbits, Guinea pigs, dogs and monkeys), independent of the route of exposure. Key effects included drastic reductions in red blood cell (RBC) counts, haemoglobin (Hb) concentrations and haematocrit (HCT). Based on these results, the DS proposed classification of 2-butoxyethanol as STOT RE 2; H373 (May cause damage to blood through prolonged or repeated exposure).

Comments received during public consultation

One MSCA disagreed with the proposed classification as STOT RE 2. According to this MSCA, 2-butoxyethanol caused marked haemolysis in 3 commonly used animal test species (mouse, rat and rabbit). However, the MSCA noted that there is compelling evidence that humans and other test species such as Guinea pig are remarkably resistant to this effect. The classification criteria state that findings in animals should be of relevance to human health, and as that is not the case for 2-butoxyethanol, the MSCA said that the substance should not be classified for STOT RE, based on haemolysis seen after repeated exposure.

Another MSCA noted that studies with very short duration should not be taken into account in classification for STOT RE. For 2-butoxyethanol, studies with longer exposure would lead to a less severe classification or no classification at all. The MSCA commented that this should have been discussed in the CLH proposal.

One industry or trade association disagreed with the proposed classification for STOT RE, saying that the main and most significant substance-specific toxic effect of 2-buthoxyethanol is haemolysis, in some cases at low doses, but that this effect it is also highly species-specific and that that rodents and rabbits are particularly susceptible to haemolysis caused by 2-butoxyethanol exposure, while there is compelling evidence showing that humans and some other test species, such as the Guinea pig, are remarkably resistant.
They also commented that the haemolysis of RBCs is caused by butoxy acetic acid (BAA), the primary metabolite of 2-butoxyethanol, stating that this metabolite is produced by the alcohol and aldehyde dehydrogenase enzyme system in the liver. Rodents, Guinea pigs and humans are all capable of converting 2-butoxyethanol to BAA; however, 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 RBC haemolysis using rodents, rabbits, Guinea pigs, cats, dogs, pigs and primates (including humans) has demonstrated that the RBCs of rodents and rabbits are considerably more sensitive to this effect, whereas Guinea pigs and humans are significantly less sensitive.

A study looking at potentially susceptible sub-populations of humans, found that that none of the populations showed any susceptibility to BAA-induced haemolysis. Pharmacokinetic models have demonstrated that it is not possible to achieve a high enough plasma concentration of BAA in humans by inhalation or dermal routes to trigger even a slight haemolysis of RBC.

The commenting party further said that BAA appears to increase the fragility of RBCs in some species, leading them to rupture when passing through the vascular system; and also produces more haemolysis in older animals compared to younger animals, due to the increased fragility of older RBCs (present in higher numbers in old animals).

The commenting party further said that there is a number of accidental poisoning case reports in humans with exposure to 2-butoxyethanol at very high doses and that the majority of these reports show no evidence of haemolysis, even at very high doses. Similarly, there are volunteer studies where no haemolysis was seen at doses which would have caused severe haemolysis in rodents or rabbits.

They also summarised several mechanistic studies exploring the mode of action of 2-butoxyethanol.

To support their view, the industry or trade association also quoted opinions from several bodies, including e.g. the United Nations Committee of Experts, the US EPA and IARC, all recognising

the large species differences in sensitivity between rats/mice/rabbits and humans. They also referred to the EU risk assessment of 2-butoxyethanol, where 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, recognising that humans are far less sensitive to the haemolytic effects of 2-butoxyethanol than rats, mice and rabbits (EU RAR, 2006).

The DS in their response appreciated that the consortium agreed that 2-butoxyethanol causes marked haemolysis in various animal species (e.g. mice, rats, rabbits). The DS further noted 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 2-butoxyethanol in most mammals. The proposed differences between species were suggested by the DS to be due to the slower metabolic rate and the lower percentages of 2-butoxyethanol being converted to BAA in humans versus e.g. rats, as well as the lower susceptibility of human erythrocytes compared to rat erythrocytes to BAA effects *in vitro*. 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. The DS said that this indicates that haemolysis due to 2-butoxyethanol exposure cannot only be due 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 RBC is only caused by BAA.

Further, the DS commented on the *in vitro* studies showing resistance of RBCs of humans and Guinea pigs to BAA noting that this conclusion is based on using blood cells of numerous species, including humans. Udden (1994a,b and 2002) and Ghanayem and Sullivan (1993) have both done *in vitro* test with RBCs from various species, including humans, and found that species difference do exist. However, the DS highlighted that *in vitro* studies do not necessarily reflect *in vivo* conditions, but can rather 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.

The DS further noted that, although that in some of poisoning and accidental exposure case reports on 2-butoxyethanol in humans, at very high doses, no evidence of haemolysis following exposure was reported, in other cases severe haemolytic effects were observed. Further, as these were case studies, not necessarily all parameters of interest were identified/measured. Moreover, human subjects were exposed to 2-butoxyethanol acutely, i.e. only once, and consequences of a repeated or chronic human exposure have not been assessed.

In response to the industry or trade association comment that "*there is no evidence to suggest that chronic exposure will produce worse effects than short term exposure*" the DS noted that the assumption that haemolytic effects due to 2-butoxyethanol exposure diminish over time is based on the results from a study in male rats, whereas no evidence in humans is available. In follow-up studies in rats, it was noted that acute exposure (daily for 1-3 days) lead to an increase in haemolysis of erythrocytes, but that the number of erythrocytes began to rebound when exposure was continued, approaching pre-treatment levels within 12 days, suggesting development of tolerance to the haemolytic effect of 2-butoxyethanol. The DS commented that this may be partly attributable to compensation mechanisms due to increased erythropoiesis and considered it uncertain whether a true tolerance mechanism (resulting in a lower degree of haemolysis) was detected.

Regarding the comment on study duration, the DS highlighted that also longer term studies (14-90 days), showed effects warranting classification as STOT RE 2. The DS further agreed with the consortium that chronic exposure to 2-butoxyethanol may not produce more severe effects than short term exposure due to compensation mechanisms or a potential decrease in susceptibility of newly formed erythrocytes after the occurrence of the anaemia. Nevertheless, the DS were of the opinion that Haber's rule can be applied, especially in view of the above mentioned findings in the long-term studies.

The DS also noted that the high inter-individual variation in permeation, absorption and elimination of 2-butoxyethanol detected in studies performed on human volunteers are of high relevance for the classification of 2-butoxyethanol.

In summary, the DS concluded that 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, and the variety of mammalian species which are severely affected by exposure to this chemical (including humans), as well as the remaining uncertainty whether BAA is the only responsible metabolite for the haemolytic effects, classification as STOT RE 2 is warranted for 2-butoxyethanol.

Assessment and comparison with the classification criteria

For 2-buthoxyethanol there is a considerable number of studies in which this substance was administered by the oral, dermal, inhalation or subcutaneous route, with exposure times varying from 3 to 90 or even more days. Unfortunately, in some studies the effects were not sufficiently described or quantified; therefore, although they provide information on type of effects induced by 2-buthoxyethanol, their results cannot be used for comparison with the classification criteria.

These studies demonstrate that the most sensitive cells are erythrocytes where 2-buthoxyethanol induces cell swelling and haemolysis, leading to premature destruction of erythrocytes, reduction of Hb level in blood, increased medullary and extramedullary haematopoiesis, increased percentage of reticulocytes in blood, and deposition of hemosiderin in spleen, liver and kidney with eventual fibrosis of these organs. The reduction in Hb, RBCs and HCT are typical symptoms of hemolityc anemia, which is an adverse but reversible effect. It should be used for classification if it is severe or when there is clear evidence for marked organ dysfunction. According to Muller *et al*. (2006; providing guidance on hazard classification of chemicals inducing haemolytic anaemia), a reduction in Hb alone at or above 20%, or a reduction of Hb at or above 10% together with indicators of dysfunctions or organ damage, justify classification.

Since 90-d studies are considered to be most appropriate for assessment of repeated dose toxicity, a comparison of the observed effects with classification criteria starts with studies of that duration.

90 days or longer studies

Guidance values (GVs) for 90-d studies for STOT RE can be found in Annex I to CLP, tables 3.9.2 and 3.9.3. For studies of greater or lesser durations, GVs have been extrapolated using Haber's rule (see 3.9.2.9.5, Annex I, CLP).

Oral exposure

1. In a 90-d NTP study (1993; OECD TG 408, GLP), rats were given 2-buthoxyethanol daily in drinking water at concentrations of 0, 750, 1500, 3000, 4500, 6000 ppm (equivalent

to 0, 82, 151, 304, 363, 470 mg/kg bw/d for females; 0, 69, 129, 281, 367, 452 mg/kg bw/d for males). Reduced RBC count and Hb concentration were observed at exposure level ≥ 69 – 82 mg/kg bw/d (females) and ≥ 129 – 151 mg/kg bw/d (males). A reduction in RBC above 10% was noted at 281 mg/kg bw/d after 1 week of exposure and at 129 mg/kg bw/d after 13 weeks. A reduction of Hb above 10% was noted at 281 mg/kg bw/d after 1 week of exposure and at 452 mg/kg bw/d after 13 weeks. Prolongation of exposure from 1 week to 13 weeks did not increase the severity of haematological effects; rather the opposite as higher dose levels were required to induce reduction of Hb concentration above 10% after 13 weeks of exposure than after 1 week, indicating adaptation to the 2-buthoxyethanol induced toxicity over time. Liver lesions were seen at all doses: cytoplasmic alteration (≥ 69/89 mg/kg bw/d), eosinophilic, hepatocellular degeneration (≥ 281/304 mg/kg bw/d), pigmentation of Küpffer cells (≥ 129/151 mg/kg bw/d), hyperplasia of bone marrow (≥ 281/304 mg/kg bw/d), increased haematopoiesis and haemosiderin pigmentation in the spleen (≥ 129/151 mg/kg bw/d, linear dose-response).

Conclusion: It is noted that severe symptoms of haemolytic anaemia were observed in the 90-d oral toxicity study, but at dose level above the GVs for STOT RE 2. No reduction of Hb above 10% was noted at dose level of 69 – 151 mg/kg bw/d indicating that the haemolytic effects at these dose levels do not meet the classification criteria. In addition, the hepatocellular degeneration, pigmentation of Küpffer cells, hyperplasia of bone marrow, increased haematopoiesis and haemosiderin pigmentation in the spleen occurred above the GVs for STOT RE 2. Therefore, the results of this 90-d study does not meet the classification criteria for STOT RE 2.

2. In a 90-d study (Siesky *et al*., 2002; non-guideline, non-GLP) in rats given 225 or 450 mg/kg bw/d by gavage, decreased HCT were seen at all time points (approx. -15% at both concentrations after 90 days), and significantly higher spleen weight were seen at all doses already after 7 days. No information on Hb and RBC was provided. A dose and time related significant increase of Perl's index in Küpffer cells (hepatic deposition of iron; 2 to 10 folds at 225 mg/kg bw/d and 4 to 25 folds at 450 mg/kg bw/d) were seen, and assumed to indicate haemosiderin deposition following haemolysis.

Conclusion: It is noted that some symptoms of haemolytic anaemia were observed in the 90-d oral toxicity study in rats but at dose levels above the GVs for STOT RE 2.

3. In a non-guideline, non-GLP, repeated-dose toxicity study (Siesky *et al.*, 2002) mice were given 2-buthoxyethanol by gavage at doses 0, 225, 450, 900 mg/kg bw, 5 d/week for 7, 14, 28 or 90 days. A significantly decreased HCT (-13, -6, -8, -13% at 450 mg/kg bw/d and -20, -9, -15, -17% at 900 mg/kg bw/d after 7, 14, 28 and 90 days) was noted, showing that the reduction of HCT was not increasing with time at any dose level. Significantly increased spleen (~2-fold) and liver (~1.2-fold) weights were seen at 450 and 900 mg/kg bw/d already after 7 days. A dose and time related increase in Perl's index in Küpffer cells (hepatic deposition of iron; 4- to 14-fold at 450 mg/kg bw/d and 14 to 28-fold at 900 mg/kg bw/d) were seen.

Conclusion: It is noted that some symptoms of haemolytic anaemia, as well as changes in liver and spleen weight, were observed, but at dose levels above the GVs for STOT RE 2. No haematotoxic effect, other than a reduction in HCT, were reported after exposure for 90 days at 225 mg/kg bw/d, suggesting that they were not observed at that dose.

Summary conclusion of 90-d oral toxicity studies

In the oral 90-d repeated toxicity studies summarised above, the dose levels were above GVs for STOT RE 2, and/or the results were not presented in sufficient detail to compare with the classification criteria. Therefore, the results of the oral 90-d studies do not justify classification of 2-buthoxyethanol as STOT RE 2.

Inhalation exposure

For comparison with the classification criteria for STOT RE 2, the following GVs were used:

- $0.2 < C \leq 1.0$ mg/L/6h/d, duration 3 months (90 days)
- $0.1 < C \leq 0.5$ mg/L/6h/d, duration 6 months
- $0.05 < C \leq 0.25$ mg/L/6h/d, duration 12 months
- $0.03 < C \leq 0.13$ mg/L/6h/d, duration 24 months

4. In a non-guideline, non-GLP study (Anonymous, 1970), rats were exposed by inhalation (vapour, whole body) to 2-buthoxyethanol at concentration of 0.24 mg/L (50 ppm), 7 h/d, 5 d/week for 90 days. An increase in erythrocyte osmotic fragility, statistically significant at study end (+30%) were seen, as well as a significantly increased relative (but not absolute) kidney weight (+6.2%). No further details were reported.

Conclusion: The level of exposure was in the range of GVs for STOT RE 2, but the data was not described in sufficient detail (no data on Hb and RBC) to allow a comparison with the classification criteria.

5. In a study similar to OECD TG 413 (Subchronic Inhalation Toxicity, with some deviations; 90-d; GLP-compliance not specified but assumed; Bushy Run Research Center, 1981b) rats were exposed by inhalation (vapour) to 2-buthoxyethanol at concentrations of 0, 0.02, 0.12 and 0.37 mg/L (0, 5, 25, 77 ppm), with an exposure duration of 6 h/d, 5 days/week for 42 or 90 days. No mortality but transient decrease in body weight gain was seen at 0.37 mg/L. Significant haematological effects at 0.37 mg/L (with effects greater after 6 weeks than at 13 weeks) were decreases in RBC count, Hb concentration, HCT and an increase in mean corpuscular haemoglobin (MCH). No quantitative details were reported; however, RBC, Hb and HCT were not significantly affected at concentrations of 0.02 and 0.12 mg/L.

Conclusion: The level of exposure was within the GVs for STOT RE 2, but the data was not described in sufficient detail (no data on Hb, HCT and RBC) to allow a comparison with the classification criteria.

6. In another study similar to OECD TG 413 (GLP-compliance not specified; Dodd *et al*., 1983) male and female rats were exposed by inhalation (vapour, whole body) to 2-buthoxyethanol at concentrations of 0, 0.02, 0.12, 0.37 mg/L (0, 5, 25 or 77 ppm) for 6 h/d, 5 d/week for 90 days.

The following effects were observed:

- significant decrease in RBC counts (up to -13% at 0.37 mg/L);
- significant decrease in Hb concentrations in females at 0.37 mg/L (no details reported);
- significant decrease in HCT in females at 0.37 mg/L (no details reported);
- significant increase in females MCH (+11%) at 0.37 mg/L.

These effects were noted at interim sacrifice and persisted throughout the study without increase in severity. At the end of the 90-d study, haematologic effects either decreased or returned to

control ranges; no longer statistically significant. There was no treatment related alteration in erythrocyte fragility at either interim or terminal sacrifice. No other haematological findings of toxicological significance were observed among the rats. There was no indication of RBC or Hb in the urine collected daily during the first exposure week, or weekly thereafter, from the male and female rats at the high exposure level. No urinary findings of toxicological significance were observed among the rats. Several incidental lesions were present in various organs, however no treatment-related, gross or microscopic lesions were found in either the male or female rats sacrificed at the end of the study.

Conclusion: The dose levels of 0.02-0.37 mg/L were within the GVs for STOT RE 2, but the severity of haematological effects in females seen after 90 days of exposure was not sufficient to meet the classification criteria; no effects were seen in males. No pathological changes were seen in liver, spleen or other organs. Therefore, it is concluded that the observed effects do not meet criteria for classification as STOT RE 2.

7. In an OECD TG 453 (Combined chronic toxicity/carcinogenicity study, GLP-compliant; NTP, 2000) rats (50/sex/dose) were exposed by inhalation (vapour, whole body) to 2-buthoxyethanol at concentrations of 0, 0.15, 0.3, 0.6 mg/L (31, 62.5, 125 ppm). The exposure duration was 6 h/d plus chamber equilibration time (12 min), 5 d/week, for 3, 6, 12 and 24 months.

Survival of treated rats was similar to the controls. There was a decreased body weight in females at 0.6 mg/L. Haematological examination showed that inhalation of 2-butoxyethanol resulted in the development of a persistent and exposure related macrocytic, normochromic, responsive anaemia, as indicated by decreased HCT values, Hb concentrations and erythrocyte counts. These changes occurred at 3, 6 and 12 months in 62.5 ppm group females and 125 ppm group in males. Some anaemia also occurred at 3 and 6 months in the 31.2 ppm group females and at 12 months in the 62.5 ppm group males. In females this was characterised by a dose-related and significant fall in HCT, Hb and erythrocyte count and an increase in MCV. The changes at 31 ppm were however small (< 5%).

Increases in circulating reticulocyte and nucleated erythrocyte counts are consistent with an erythropoietic response to the anaemia. Increases in bone marrow cellularity occurred at all time points in females at 125 ppm along with a 15–35% decrease in M/E ratio. Significant changes were also seen in males at 125 ppm and females at 62.5 ppm but only at one time point. The severity of the response was dose related.

Haematotoxicity (values after 24 months not reported):

- significant decrease in RBC counts after 3, 6, and 12 months at ≥ 0.3 mg/L in females and 0.6 mg/L males, as well as after 3 and 6 months at 0.15 mg/L in females and after 12 months at 0.6 mg/L in males (> 10% at 0.3 mg/L after 6 months and at 0.6 mg/L after 12 months; max. -16% in males and -14% in females);
- significant decrease in HCT after 3, 6, and 12 months at ≥ 0.3 mg/L in females and 0.6 mg/L in males, as well as after 3 and 6 months at 0.15 mg/L in females and after 12 months at 0.6 mg/L in males (max. -10% in males and -13% in females; -10% in females after 6 months at 0.15 mg/L);
- significant decrease in Hb concentration after 3, 6, and 12 months at ≥ 0.3 mg/L in females and 0.6 mg/L males, as well as after 3 and 6 months at 0.15 mg/L in females after 12 months at 0.6 mg/L in males (> 10% at 0.15 mg/L in females after 6 months and at 0.6 mg/L in both sexes after 12 months; max. -12% in males and -13% in females);

- macrocytosis: significant increase in MCV after 3, 6, and 12 months at ≥ 0.3 mg/L in both sexes and after 3 months at 0.15 mg/L in both sexes;
- significant increase in MCH after 3, 6, and 12 months at ≥ 0.6 in males and ≥ 0.3 mg/L in females;
- significant increases in reticulocytes in females (at 0.3 mg/L) and males (at 0.6 mg/L).

Cytological, morphologic alterations and megakaryocytes were present in all exposure groups.

Histopathologic effects (after 2 years):
- significantly increased hyaline degeneration of the olfactory epithelium in males at all concentrations;
- Küpffer cell pigmentation in liver in both sexes at ≥ 0.3 mg/L (linear dose-response);
- spleen fibrosis in males at ≥ 0.3 mg/L.

The following adverse haematotoxic effects were seen:

after 90 days of exposure
- an Hb decrease of > 10% was not reported for males and females;
- an RBC count decrease of > 10% was not reported for males and females;
- an HCT decrease of > 10% was not reported for males and females.

after 6 months of exposure
- Hb concentration decrease of > 10% at 0.15 mg/L in females;
- RBC decrease of > 10% at 0.3 mg/L;
- HCT decrease of > 10% in females at 0.15 mg/L.

after 12-24 months of exposure
- Hb decrease of > 10% at 0.6 mg/L in both sexes after 12 months (max. -12% in males and -13% in females);
- RBC decrease of > 10% at 0.6 mg/L after 12 months (max. -16 % in males and -14 % in females);
- HCT significant decrease after 12 months at ≥ 0.3 mg/L in females and 0.6 mg/L males (max. -10% in males and -13% in females).

There were no histopathological examinations after 6 or 12 months. After 24 months of exposure an increase in incidences of Küpffer cell pigmentation of the liver was observed in all exposed groups of male rats (chamber control, 23/50; 0.15 mg/L, 30/50; 0.3 mg/L, 34/50; 0.6 mg/L, 42/50) and in the 2 higher exposure groups of female rats (chamber control, 15/50; 0.15 mg/L, 19/50; 0.3 mg/L, 36/50; 0.6 mg/L, 47/50). The severity of the lesion increased in the 0.6 mg/L group of both sexes.

The results of the study indicate that inhalation exposure to 2-buthoxyethanol for 3, 6, 12 and 24 induced haemolytic anaemia, although the decrease of Hb alone was not sufficiently high (< 20%) to justify classification as STOT RE 2. The haematotoxic effects seen after 3 months of exposure at 0.15, 0.3, 0.6 mg/L (GVs: 0.1 < C ≤ 0.5 mg/L/6h/d) were not severe enough to justify classification since the Hb decrease alone were below 10% (according to the guidance on hazard classification of chemicals inducing haemolytic anaemia by Muller *et al*.; 2006).

It is not possible to establish whether the small decrease in Hb concentration (but still > 10%) observed after 6 months of exposure at concentration of 0.15 mg/L in females was accompanied by relevant organ (liver, spleen) damage or dysfunction. Hence, it is not known whether relevant

damage of dysfunction of these organs occurred or not, and consequently not possible to conclude if this observation meets the criteria for STOT RE 2. The increased incidences of Küpffer cell pigmentation of the liver in male (chamber control, 23/50; 0.15 mg/L, 30/50) and female rats (chamber control, 15/50; 0.15 mg/L, 19/50) were not statistically elevated.

Thus, since the results of this study are not sufficiently reported after 6 months of exposure it is not possible to conclude whether the effects meet the classification criteria for STOT RE 2.

The haematotoxic effects (Hb and RBC decreases of more than 10%) after 12 months of exposure at concentrations ≥ 0.3 mg/L were only observed at concentrations above the GVs (0.05 < C ≤ 0.25 mg/L/6h/d), thus they do not justify classification.

Conclusion: The findings in this study does not seem to meet the classification criteria for STOT RE 2.

8. In a non-guideline, non-GLP, repeat dose toxicity study (Nyska *et al*., 1999), rats were exposed by inhalation (vapour, whole body) to 2-buthoxyethanol at concentrations of 0, 0.15, 0.3, 0.6, 1.2, 2.4 mg/L (0, 31, 62.5, 125, 250, 500 ppm), for 6 h/d, 5 d/week for 13 weeks.

Effects observed:
- 5/10 females killed moribund at 2.4 mg/L.

Haematotoxicity (at 2.4 mg/L):
- macrocytic, normochromic, and regenerative anaemia (no details reported);
- disseminated thrombosis involving coccygeal vertebrae, cardiac atrium, lungs, liver, pulp of incisor teeth, and submucosa of anterior section of nasal cavity.

Pathological and other effects (at 2.4 mg/L):
abnormal breathing, pallor, red urine, lethargy;
- coccygeal vertebral changes consistent with bone infarction in females;
- transient or complete bone growth arrest in females;
- diffuse growth plate degeneration of vertebrae, no evidence of renewed longitudinal growth;
- ischemic necrosis and/or degeneration of bone marrow cells, bone-lining cells, osteocytes (within cortical and trabecular bone), and chondrocytes (both articular and growth plate), extended to growth plate, capping of growth plate with a dense layer of bone;
- secondary foreign body-type inflammation, extended to the growth plate;
- atrophy of the spleen and thymus;
- inflammation, necrosis, ulceration, and hyperplasia of the forestomach;
- centrilobular degeneration of the liver;
- haemoglobinuric nephrosis.

No details on effects at < 2.4 mg/L were reported.

Conclusion: There is a lack of details reported for effects seen within the GVs for STOT RE 2 (0.2 < C ≤ 1.0 mg/L/6h/d, 90-d study).

9. In a non-guideline, non-GLP, repeat dose toxicity study (Long *et al*., 2000), female rats were exposed by inhalation (vapour, whole body) to 2-buthoxyethanol at concentrations

of 0, 0.15, 0.3, 0.6, 1.2, 2.4 mg/L (0, 31, 62.5, 125, 250, 500 ppm), 6 h/d, 5 days/week for 13 weeks.

Effects observed:
- 5 out of 10 females of the 2.4 mg/L group (4/5 on day 4; 1 on day 32) and 1/10 rats from the 1.2 mg/L group (during week 8) were killed moribund (due to haematologic alterations).

Haematotoxicity:
- significant decrease in RBC counts at ≥ 0.15 mg/L (> 10% at 0.3 mg/L);
- significant decrease in HCT counts at ≥ 0.15 mg/L (> 10% at 0.6 mg/L);
- significant decrease in Hb concentration at ≥ 0.15 mg/L (> 10% at 0.6 mg/L; > 20% at 1.2 mg/L);
- significant increase in reticulocytes, MCV, MCH and platelet concentration at ≥ 0.6 mg/L.

Microscopic changes in maxillary incisors after 4 days at 2.4 mg/L:
- thrombosis of pulp blood vessels;
- multifocal necrosis of pulp stroma;
- multifocal necrosis of odontoblasts;
- some thrombosed blood vessels developed fibrinoid degeneration of the vessel wall;
- acute haemorrhage within the surrounding dental pulp;
- acute and abrupt coagulative necrosis of multiple segments of odontoblasts underwent
- degenerative changes in ameloblast layers.

No thrombosis or degeneration seen at 1.2 mg/L.

Conclusion: The results of the study indicate that inhalation exposure to 2-buthoxyethanol at concentrations of 0.15, 0.3, 0.6, 1.2, 2.4 mg/L for 13 weeks induced haemolytic anaemia. However, the decrease of Hb alone after exposure within GVs (0.2 < C ≤ 1.0 mg/L/6 h/d) was not sufficiently high (< 20%) to justify classification as STOT RE 2.

10. In an OECD TG 413 (Subchronic Inhalation Toxicity, 90-d study, GLP compliant; NTP, 2000) rats were exposed by inhalation (vapour, whole body) to 2-buthoxyethanol at concentrations of 0, 0.15, 0.3, 0.6, 1.2, 2.4 mg/L (0, 31, 62.5, 125, 250, 500 ppm), 6 h/d plus chamber equilibration time (12 min), 5 d/week for 14 weeks.

Effects observed:
- 6/10 females killed moribund (1/10 at 0.6 mg/L during week 8, 4/10 at 2.4 mg/L during week 5, 1 /10 at 2.4 mg/L during week 5);
- abnormal breathing, pallor, red urine stains, nasal and eye discharge, lethargy, and increased salivation and/or lacrimation at ≥ 0.6 mg/L, most prevalent during the first 2 weeks of exposure;
- significantly increased kidney weight (males at 2.4 mg/L; females at ≥ 0.6 mg/L) and liver weight (males at ≥ 1.2 mg/L; females at ≥ 0.6 mg/L);
- significantly reduced thymus weights of females at 2.4 mg/L

Haematotoxicity at ≥ 0.6 mg/L in males and at ≥ 0.15 mg/L in females:
- significant decrease in RBC counts (> 10% at ≥ 0.6 mg/L; max. -34% in males and -44% in females at 2.4 mg/L);
- significant decrease in HCT (max. -21% in males and -25% in females at 2.4 mg/L);

- significant decrease in Hb concentration (> 10% at ≥0.6 mg/L; max. -25% in males and -33% in females at 2.4 mg/L; females: -4, -6 and -13% at 0.15, 0.3 and 0.6 mg/L, respectively; males: -7% at 0.6 mg/L);
- significant increase in reticulocytes (in females at ≥ 0.6 mg/L);
- significant increase in nucleated erythrocytes (in females at ≥ 0.3 mg/L, in males at ≥ 1.2 mg/L);
- significant increase in MCV and MCH (in females at ≥ 0.3 mg/L);
- leukocytes: decreased lymphocyte and monocyte counts only in males at ≥ 1.2 mg/L.

Histopathologic effects at ≥ 1.2 mg/L for males and ≥ 0.6 mg/L for females:
- spleen atrophy, excessive splenic congestion due to extramedullary haematopoiesis;
- haemosiderin accumulation/ pigmentation in Küpffer cells (in males already at 0.6 mg/L; in females already at 0.3 mg/L);
- liver necrosis and centrilobular degeneration;
- renal tubular degeneration and pigmentation (intracytoplasmic haemosiderin deposition);
- bone marrow hyperplasia (in females already at 0.3 mg/L);
- inflammation, necrosis, and ulceration of forestomach (only in males);
- tail necrosis in females (only at 2.4 mg/L).

Conclusion: The results of the study indicate that inhalation exposure to 2-buthoxyethanol at concentrations of 0, 0.15, 0.3, 0.6, 1.2, 2.4 mg/L for 14 weeks induced haemolytic anaemia, although the decrease of Hb alone after exposure within GVs (0.2 < C ≤ 1.0 mg/L/6h/d) was not sufficiently high (< 20%) to justify classification as STOT RE 2. However, a smaller decrease in Hb concentration (> 10%) observed after 14 weeks of exposure at concentration of 0.6 mg/L in females was accompanied by changes in relevant organs (liver, spleen, bone marrow) indicating organ damage or dysfunction. Hence, the criterion for classification as STOT RE was met in this study.

11. In a GLP-compliant OECD TG 413 (Subchronic Inhalation Toxicity, 90-d; NTP, 2000) study, mice were exposed by inhalation (vapour, whole body) to 2-buthoxyethanol at concentrations of 0, 0.15, 0.3, 0.6, 1.2, 2.4 mg/L (0, 31, 62.5, 125, 250, 500 ppm), 6 h/d plus chamber equilibration time (12 min), 5 d/week for 14 weeks.

Effects observed:
- at 2.4 mg/L, 2 males and 2 females were killed moribund during the first 2 weeks. The animals showed abnormal breathing, red urine stains and lethargy;
- significant lower body weight and body weight gains at ≥ 0.6 mg/L;
- increase in relative liver weights at 1.2 mg/L in males and 2.4 mg/L in females

Haematotoxicity:
- significant decrease in RBC counts at 1.2 mg/L in males and 0.6 mg/L in females (> 10% at 1.2 mg/L; max. -26% in males and -24% in females at 2.4 mg/L);
- significant decrease in HCT at 0.6 mg/L in males and 0.15 mg/L in females (max. -26% in males and -24% in females at 2.4 mg/L);
- significant decrease in Hb concentration at 0.6 mg/L in males and 0.15 mg/L in females (> 10% at ≥ 1.2 mg/L in females; max. -27% in males and -24% in females);
- significant increase in reticulocytes at 0.6 mg/L in both sexes (3.7-fold in males and 6.5-fold in females);
- significant increase in MCH in females at ≥ 2.4 mg/L;
- significant increase in platelets at 2.4 mg/L in males and 1.2 mg/L in females;
- increased numbers of polychromatophilic erythrocytes

Histopathologic effects:

- lymphoid atrophy of the spleen, thymus, and mesenteric and mandibular lymph nodes in males and females at 2.4 mg/L;
- renal cortical degeneration and some necrosis (glandular eosinophilic debris in the lumen of the cortical tubules and pyknotic nuclei) at 2.4 mg/L;
- testicular degeneration and necrosis of the epididymis at 2.4 mg/L;
- epithelial hyperplasia and inflammation of the muscularis or serosa of the forestomach in females at ≥ 0.6 mg/L;
- minimal to mild forestomach inflammation at 2.4 mg/L;
- extramedullary haematopoietic cell proliferation, primarily erythroid, and haemosiderin pigmentation of the spleen in males at ≥ 0.6 mg/L and in females at ≥ 1.2 mg/L;
- haemosiderin pigmentation in Küpffer cells in males at 2.3 mg/L and females at ≥ 1.2 mg/L;
- renal tubule haemosiderin pigmentation in males and females at 2.4 mg/L.

Conclusion: The results of the study indicate that inhalation exposure of mice to 2-buthoxyethanol at concentrations 0, 0.15, 0.3, 0.6, 1.2, 2.4 mg/L, 5 d/week for 14 weeks induced haemolytic anaemia, although the decrease of Hb alone after exposure within GVs ($0.2 < C \leq 1.0$ mg/L/6h/d) was not sufficiently high (< 20%) to justify classification as STOT RE 2. A smaller decrease in Hb concentration, but above 10%, was observed after 14 weeks of exposure at concentrations ≥ 1.2 mg/L, accompanied by changes in relevant organ (spleen, kidneys) indicating damage or dysfunction This was however observed only above the GVs for STOT RE 2. Therefore, it is concluded that the classification criteria for STOT RE 2 are not met.

12. In a non-guideline, not GLP-compliant, repeat dose toxicity study (Mellon Institute of Industrial Research, 1956, cited in Carpenter *et al*., 1956), male mice were exposed by inhalation (vapour, whole body) to 2-buthoxyethanol at concentrations of 0, 0.48, 0.97, 1.93 mg/L (0, 100, 200, 400 ppm), 7 h/d, 5 d/week for 90 days. The post exposure period was 42 days.

No mortality was observed.

Haematotoxicity:

- haematuria at all concentrations (linear dose-response; recovered after 3 exposures);
- significant increase in erythrocyte fragility (recovered 17 h post exposure);
- significantly increased liver weights at 1.93 mg/L (recovered within 42 days post exposure);

Conclusion: The results of the study indicate that inhalation exposure of mice to 2-buthoxyethanol for 90 days induced haemolytic anaemia. However, insufficient reporting of the results does not allow a comparison with the classification criteria. It is noted that haematuria was observed only during the first 3 exposure days, indicating acute toxicity of the substance.

13. In an OECD TG 453 (Combined chronic toxicity/carcinogenicity study, GLP-compliant; NTP, 2000), mice were exposed by inhalation (vapour, whole body) to 2-buthoxyethanol at concentrations of 0, 0.3, 0.6, 1.2 mg/L (0, 62.5, 125, 250 ppm), 6 h/d plus chamber equilibration time (12 min), 5 d/week for 3, 6, 12 and 24 months.

Effects observed:

- significantly higher mortality of male mice at ≥ 0.6 mg/L (no details reported);

- significantly lower body weights of females (during the whole study) and males (during the last 6 months) at ≥ 0.3 mg/L.

Haematotoxicity (values after 24 months not reported):
- significant decrease in RBC counts after 3, 6, and 12 months at ≥ 0.6 mg/L in both sexes, as well as after 6 months at 0.3 mg/L in females (> 10% at 1.2 mg/L after 6 months and at 1.2 mg/L after 12 months; max. -13% in both sexes);
- significant decrease in HCT after 3, 6, and 12 months at ≥ 0.6 mg/L in both sexes, as well as after 6 months at 0.3 mg/L in females (max. -13% in males and -11% in females);
- significant decrease in Hb concentration after 3, 6, and 12 months at ≥ 0.6 mg/L in both sexes, as well as after 6 months at 0.3 mg/L in females (> 10% at 1.2 mg/L after 6 and 12 months);
- significant increases in reticulocytes in males and females at ≥ 0.6 mg/L;
- changes in MCV only in females after 12 months at 1.2 mg/L;
- no changes in MCH and MCH concentration (MCHC; mean Hb concentration in the RBC);
- thrombocytosis (increase in platelet counts) at 1.2 mg/L in males and females after 3, 6, and 12 months, as well as in females at ≥ 0.3 mg/L after 6 months and in both sexes at 0.6 mg/L after 12 months;
- increased neutrophil counts at 0.6 mg/L in both sexes at 6 months and at ≥ 0.6 mg/L in females after 12 months.

Histopathologic effects:
- incidences of haemosiderin pigmentation in Küpffer cells of the liver significantly increased in males of the 0.6 and 1.2 mg/L groups and females of all groups exposed in a dose-dependent manner;
- haematopoietic cell proliferation in the spleen increased in males at 0.6 and 1.2 mg/L and females at 1.2 mg/L, but was not accompanied by any change in M/E cell ratio;
- incidences of haemosiderin pigmentation in the spleen significantly increased in all exposed groups of males and at 0.6 and 1.2 mg/L in females; attributed to primary haemolysis, followed by regenerative hyperplasia of the haematopoietic tissue;
- increases in the incidence of hyperplasia were also observed in the bone marrow of males exposed to 0.6 and 1.2 mg/L;
- hyaline degeneration in olfactory and respiratory epithelium in females at all concentrations;
- glomerulosclerosis and hydronephrosis in males at ≥ 0.6 mg/L.

Haematotoxic effects:
after 3 months of exposure
- an Hb decrease of > 10% was not reported for males or females;
- an RBC count decrease of > 10% was not reported for or and females;
- an HCT decrease of > 10% was not reported for males and females.

after 6 and 12 months of exposure
- Hb concentration decrease of > 10% at 1.2 mg/L after 6 and 12 months;
- RBC decrease of > 10% at > 1.2 mg/L after 6 months and at 1.2 mg/L after 12 months (max. -13% in both sexes)

There were no histopathological examinations after 6 or 12 months. After 24 months of exposure there were changes in spleen and liver demonstrating dysfunction of these organs after exposure to 0.6 or 1.2 mg/L.

Conclusion: The results of the study indicate that inhalation exposure of mice to 2-buthoxyethanol for 3, 6, 12 and 24 months induced haemolytic anaemia, although the decrease of Hb alone was not sufficiently high (< 20%) to justify classification as STOT RE 2. The decreases in Hb concentration (> 10%) observed after 6 and 12 months of exposure at concentration of 1.2 mg/L, although accompanied by histopathological changes in relevant organs (liver, spleen), were observed above the GVs for 6 months (0.1 < C ≤ 0.5 mg/L/6h/d), and 12 months exposure (0.05 < C ≤ 0.25 mg/L/6h/d); therefore they do not meet the classification criteria for STOT RE 2. Haematological tests were performed daily and biochemistry only in the male dog after 25 and 26 days of exposure.

14. In a non-guideline, non-GLP, repeat dose toxicity study (Mellon Institute of Industrial Research, 1956; cited in Carpenter *et al*., 1956) male and female Basenji or Wire-haired terrier dogs (1 animal/sex/group) were exposed by inhalation (vapour) to 2-buthoxyethanol at concentrations of 0, 0.48, 0.97, 1.86 mg/L (0, 100, 200 and 385 ppm), 7 h/d, 7 d/week for 8 and 28 days, respectively (at 1.86 mg/L), for 31 days (at 0.97 mg/L) or for 91 days (at 0.48 mg/L).

Effects observed:
- At 1.86 mg/L:
  - the female dog died after 8 days and the male dog after 28 days of exposure (previous symptoms: weakness, apathy, anorexia, weight loss).
- At 0.97 mg/L:
  - slight evidence of toxicity after 31 days of exposure.

Haematotoxicity:
- At 0.48 mg/L:
  - slight but significant decrease in HCT.
- At 0.97 mg/L:
  - slight but significant increase in erythrocyte fragility in both sexes at 0.97 mg/L;
  - slight but significant decrease of Hb concentration throughout the study at 0.97 mg/L.
- At 1.86 mg/L:
  - erythrocyte fragility was continuously observed in both dogs; in the male dog the score on erythrocyte fragility reached a maximum in 7 days (0.54-0.42) and decreased progressively throughout 27 days (0.32-0.20); no data on the course of fragility was given for the female dog which died on day 8.

Histopathology:
- congestion of liver and lungs at 1.86 mg/L;
- congestion of kidneys only in females

Conclusion: The dose of 0.48 mg/L for 91 days is within the GVs (0.2 < C ≤ 1.0 mg/L/6h/d), STOT RE 2, but due to the lack of details (no detailed data on Hb and RBC) it is not possible to compare them with the classification criteria. It is noted that 100% mortality at 1.86 mg/L after 28 days would meet the STOT RE 2 classification criteria (GVs: 0.6 < C ≤ 3.0 mg/L/6h/d); however, only 1 animal was exposed for that length of time.

15. In a non-guideline, non-GLP, repeat dose toxicity study (Mellon Institute of Industrial Research, 1956; cited in Carpenter *et al*., 1956) female Rhesus monkeys (1/group) were exposed by inhalation (vapour) to 2-buthoxyethanol in Test 1 at concentrations of 0, 0.48,

0.97 mg/L (0, 100, 200 ppm), 7h/d, 5 d/week for 90 days; and in Test 2 to 0.48 mg/L (100 ppm) for 10 days, then 0.97 mg/L (200 ppm) for 80 days.

In Test 2, 1 animal died of causes unrelated to treatment.

Haematotoxicity (no quantitative details reported):

- Test 1: No changes in erythrocyte fragility at 0.48 mg/L, but fragility increased at 0.97 mg/L (recovered until end of study)
- Test 2: Increase in erythrocyte fragility at ≥ 0.48 mg/L (35% after 7 exposures in females; approx. 21% after the 18th exposure;). Recovery by the end of the exposure period.

Conclusion: Exposure levels of 0.48-0.97 mg/L for 90 days were within the GVs range (0.2 < C ≤ 1.0 mg/L/6h/d) for STOT RE 2, but due to the lack of details (no detailed data on Hb and RBC) it is not possible to compare them with the classification criteria. It is noted that the erythrocyte fragility observed in monkeys in Test 2 after the 7th and 18th exposure returned to normal values at the end of exposure.

16. In a study by Werner *et al*. (1943; cited by Carpenter *et al*., 1956) dogs (of unspecified strain), 2 animals/group, were exposed by inhalation to 2-butoxyethanol at concentrations of 0 or 415 ppm for 7 h/d, 5 d/week for 12 weeks. Necropsies were performed 5 weeks post exposure; haematologic parameters were examined before, during, and after the exposure. No statistical analysis was presented. The authors concluded that exposure of dogs to 2-butoxyethanol vapours resulted in decreased Hb concentration and RBC count with increased hypochromia, polychromatophilia, and microcytosis. These haematologic effects were not severe and they were reversed 5 weeks after the end of exposure.

Conclusion: Dogs exposed for 12 weeks, 7 h/d, 5 d/week, did not have haemolytic effects meeting the classification criteria for STOT RE 2, indicating a lower sensitivity to the haemolytic effects in dogs.

Summary conclusion of 90-d inhalation studies

There are 13 repeat dose inhalation toxicity studies carried out for 90 days or longer. In 6 studies (Anonymous, 1970, in rats; Bushy Run Research Center, 1981b, in rats; Nyska *et al*., 1999, in rats; Mellon Institute of Industrial Research, 1956, 3 studies, in rats, monkey and mice, respectively) numerical data on Hb and RCB counts or histopathological findings were not provided for exposure levels within the GVs, and hence, the results cannot be compared with the classification criteria.

In one study (Long *et al*., 2000, in rats) the effects observed for exposure levels within GVs met the classification criteria for STOT RE 2 (blood). However in 6 studies (Dodd *et al*., 1983; NTP, 2000, 4 studies, 2 in mice and 2 in rats; Werner *et al*., 194, in dogs) the effects observed within GVs do not justify classification for STOT RE 2.

Dermal exposure

17. In a OECD TG 411 (subchronic dermal toxicity study; GLP, 90-d; Wil Research Laboratories, 1983) study, male and female New Zealand White rabbits (10/sex/group) were exposed via occlusive dermal application to 2-buthoxyethanol at concentrations of 0, 2.8, 14.3, 42.8% aqueous solutions (equivalent to 0, 10, 50 and 150 mg/kg bw, respectively), 6 h/d, 5 d/week for 13 weeks.

Effects seen:

- No (histo)pathological changes of organs;
- No changes in organ weight.

Haematotoxicity:

- sporadic changes in RBC counts and fragility, Hb concentration and HCT, but values were within normal ranges for the laboratory;
- red coloured faeces and red liquid material on cage paper (probably blood) in each group.

Conclusion: The results of the study demonstrate that 2-buthoxyethanol after repeated dermal occlusive application for 90 days at doses of 10, 50 and 150 mg/kg bw/d, thus within GVs for dermal exposure ($20 < C \leq 200$ mg/kg bw/d), does not produce haematotoxic or other effects fulfilling the classification criteria for STOT RE 2.

Summary conclusions of 90-d dermal toxicity studies

There is 1 repeated dermal toxicity study carried out for 90 days. The effects observed within GVs do not justify classification for STOT RE 2.

28-42 days repeat dose toxicity studies

Summary conclusion of 28/42-d *oral* toxicity studies

There are 3 repeat dose oral toxicity studies carried out for 28/42 days. In 2 studies (Kenyon *et al*., 2015; Eastman Kodak, 1982), numerical data on Hb and RCB counts or histopathological findings were not provided for exposure levels within the GVs, and therefore, the results cannot be compared with the classification criteria. In the third study (Krasavage, 1986), effects observed within GV exposure did not justify classification for STOT RE 2.

Summary conclusion of 28-42-d *inhalation* repeat dose toxicity studies

There are 3 repeated inhalation toxicity studies carried out for 28/42 days. In all these studies (Gage, 1970; Mellon Institute of Industrial Research, 1956, in rats and Guinea pigs), numerical data on Hb and RCB counts or histopathological findings were not reported for exposure levels within the GVs, and hence, the results cannot be compared with the classification criteria.

3-12 days repeated toxicity studies

Summary conclusion of 3-12 days repeat dose *oral* toxicity studies

There are 9 repeat dose oral toxicity studies in rats carried out for 3 or 4 days. The studies indicate a high haemolytic potential of 2-buthoxyethanol in rats at 250 mg/kg bw/d, corresponding to over 50% of the lowest oral LD50 for rats (470 mg/kg bw).

There are 2 repeat dose oral toxicity studies in rats and 1 in mice carried out for 7-12 days. The studies indicate a high haemolytic potential of 2-buthoxyethanol in rats and mice after several doses of 125-900 mg/kg bw/d, corresponding to 25-60% of the lowest oral LD50 for the given species.

Considering the high contribution of acute toxicity in these short term studies, their results should not be used for classification of repeat dose toxicity. There are high uncertainties whether the extrapolation of GVs for such a short exposure would be appropriate. There is a considerable number of repeat dose toxicity studies of a longer duration (28 days up to 2 years), which should be given more weight in the evaluation.

Summary conclusion of 3-12-d repeat dose *inhalation* toxicity studies

There are 3 repeat dose inhalation toxicity studies in rats or mice carried out for 10-13 days with post exposure observation periods of 6-8 days. The studies indicates high haematotoxic potential of 2-buthoxyethanol in rats after 10 days inhalation exposure at concentrations of 0.48 and 0.97 mg/L (corresponding to 25–40% of LC_{50} values in rats; the LC_{50} values in rats were 2.2 -4.92 mg/L/4h, except in one study in which an LC_{50} of 12.36 mg/L/4h was calculated) or in mice after 13 days inhalation exposure at concentrations of 0.48 and 0.97 mg/L (the highest corresponding to 23.5% of the LC_{50} in mice; the value of LC_{50} in mice was 4.12 mg/L /4h). Due to a high contribution of acute toxicity, and difficulties in comparing the reported effects with the classification criteria, the results of these studies cannot be used for classification of repeat dose toxicity; particularly taking into account that there is a considerable number of repeated dose toxicity studies in rats, mice, Guinea pigs and rabbits lasting with durations from 28 days up to 2 years which should be given more weight in the evaluation.

Summary conclusion of 3-12d repeat dose *dermal* toxicity studies

There is 1 study available, and it indicates a haematotoxic potential of 2-buthoxyethanol in rabbits; the 3 highest doses were well above the lowest LD_{50} in rabbits (LD_{50} in rabbits after 4h occlusive application were in the range of 100 - 841 mg/kg bw/d). Due to a high contribution of acute toxicity and difficulties in comparing the reported effects with the classification criteria, the results of study cannot be used for classification for repeat dose toxicity. There is also a considerable number of repeat dose toxicity studies of a longer duration (28 days up to 2 years), which should be given more weight in the evaluation.

Overall conclusion

Taking into account the results of the studies, RAC is of the opinion that 2-butoxyethanol is a strong haemolytic agent, particularly after acute and subacute exposures at lethal and sublethal dose levels. However, in the majority of the repeat oral, inhalation and dermal toxicity studies in animals carried out for 28-90 days or 1-2 years, the magnitude of the haematotoxic effects seen at exposure levels within relevant GVs were not sufficient to meet the classification criteria for STOT RE 2. RAC also took into account that the magnitude of effects seen in rodents might be higher than those in humans, since it has been demonstrated that in *in vitro* conditions RBC of rodents are more sensitive to the haemolytic action of 2-buthoxyethanl than human erythrocytes. The observations after accidental exposure or poisonings with 2-buthoxyethanol in humans do not indicate that humans are very sensitive to the haematotoxicity of this substance.

Based on this RAC is of the opinion that 2-buthoxyethanol **does not warrant classification as STOT RE 2**.

Additional references

Muller *et al*., 2006. Hazard classification of chemicals inducing haemolytic anaemia: An EU regulatory perspective. Regulatory Toxicology and Pharmacology, vol.45 (3),pp 229-241, August 2006.

EU RAR (2006): European Union Risk Assessment Report. 2-BUTOXYETHANOL. European Chemicals Bureau.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).