MCL Report for 2-butoxyethanol; ethylene glycol; monobutyl ether (EGBE)

Proposal for mandatory classification and labelling (MCL) based on Annex VI, Part 2 of the retained CLP Regulation (EU) No. 1272/2008 as amended for Great Britain

EC Number: 203-905-0

CAS Number: 111-76-2

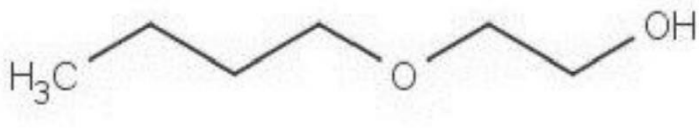
Date: August 2024

Contents

1. Identity of the substance

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	2-butoxyethanol
Other names (usual name, trade name, abbreviation)	Ethanol, 2-butoxy- (CAS name) ethylene glycol monobutyl ether (EGBE) butyl glycol
ISO common name (if available and appropriate)	*N/A*
EC number (if available and appropriate)	203-905-0
EC name (if available and appropriate)	2-butoxyethanol
CAS number (if available)	111-76-2
Other identity code (if available)	603-014-00-0 (Annex VI Index Number)
Molecular formula	$C_6H_{14}O_2$
Structural formula	
SMILES notation (if available)	OCCOCCCC
Molecular weight or molecular weight range	118.17 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	*N/A*
Degree of purity (%) (if relevant for the entry in Annex VI)	*N/A*

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current MCL on GB MCL list (if applicable)	Current self-classification and labelling (GB CLP)
2-butoxyethanol	99.5	N/A	N/A

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current MCL on GB MCL list (if applicable)	Current self-classification and labelling (GB CLP)	The impurity contributes to the classification and labelling?
N/A	N/A	N/A	N/A	N/A

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current MCL on GB MCL list (if applicable)	The additive contributes to the classification and labelling?
N/A	N/A	N/A	N/A	N/A

2. Proposed mandatory classification and labelling

Table 5: Proposed mandatory classification and labelling according to the GB CLP criteria

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	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 GB MCL list entry	603-014-00-0	2-butoxyethanol; ethylene glycol monobutyl ether	203-905-0	111-76-2	Acute Tox. 4* Acute Tox. 4 Skin Irrit. 2 Eye Irrit. 2	H332 H302 H315 H319	GHS07 Wng	H332 H302 H315 H319		oral: ATE = 1200 mg/kg bw	
Proposed Classification	603-014-00-0	2-butoxyethanol; ethylene glycol monobutyl ether	203-905-0	111-76-2	**Modify** Acute Tox. 3	**Modify** H331	**Add** GHS06 **Modify** Dgr	**Modify** H331		**Add** inhalation: ATE = 3 mg/L/4h (vapour)	
Resulting entry on the GB MCL list	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		oral: ATE = 1200 mg/kg bw inhalation: ATE = 3 mg/L/4h (vapour)	

Table 6: Reason for not proposing mandatory classification and status under public consultation

Hazard class	Classification / reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier	No
Oxidising gases	Hazard class not assessed in this dossier	No
Gases under pressure	Hazard class not assessed in this dossier	No
Flammable liquids	Hazard class not assessed in this dossier	No
Flammable solids	Hazard class not assessed in this dossier	No
Self-reactive substances	Hazard class not assessed in this dossier	No
Pyrophoric liquids	Hazard class not assessed in this dossier	No
Pyrophoric solids	Hazard class not assessed in this dossier	No
Self-heating substances	Hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	No
Oxidising liquids	Hazard class not assessed in this dossier	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not assessed in this dossier	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity via oral route	Hazard class not assessed in this dossier	No
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	**Harmonised classification proposed; Acute Tox 3 (H331; Toxic if inhaled)**	Yes
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Hazard class not assessed in this dossier	No
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Hazard class not assessed in this dossier	No
Germ cell mutagenicity	Hazard class not assessed in this dossier	No
Carcinogenicity	Hazard class not assessed in this dossier	No
Reproductive toxicity	Hazard class not assessed in this dossier	No
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	Hazard class not assessed in this dossier	No
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Hazard class not assessed in this dossier	No
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3. History of the classification and labelling

In the EU, a harmonised classification for 2-butoxyethanol was derived under Directive 67/548/EC and then translated under the CLP Regulation to: Acute Tox. 4* (oral) H302: Harmful if swallowed; Acute Tox. 4* (inhalation) H332: Harmful if inhaled; and Acute Tox. 4* (dermal) H312: Harmful in contact with skin; Skin Irrit. 2, H315: Causes skin irritation; and Eye Irrit. 2, H319: Causes serious eye irritation.

The EU Committee for Risk Assessment (RAC) reviewed the harmonised classification and labelling of 2-butoxyethanol in 2018 and concluded that the substance should be classified as Acute Tox. 3, H331; Acute Tox. 4, H302; Skin Irrit. 1, H315; Eye Irrit. 2, H319; not classified for acute dermal toxicity (ECHA, 2018).

Following industry comments and additional data submitted to CARACAL[1], RAC was asked to reassess the proposed classification for acute inhalation toxicity[2]. As such, Annex VI of EU CLP was updated by the 15th ATP to include the revised classification for 2-butoxyethanol, with the exception of acute inhalation toxicity. This revised classification was copied over into the GB MCL list when the UK left the EU.

RAC considered the new information relating to acute inhalation toxicity, which comprised of a study report from a new GLP-compliant acute toxicity inhalation study conducted according to OECD TG 433 (adapted), as well as the study report for another study in guinea pigs, rabbits and dogs, which had previously been submitted in the context of the consultation on the CLH report, but was not fully discussed in the original RAC Opinion. Following their reassessment, RAC concluded that a classification of Acute Tox 3; H331 (Toxic if inhaled), was warranted, as stated in their initial opinion. This classification was subsequently included in Annex VI of EU CLP via the 18th ATP. As this update occurred after the UK had left the EU, the revised classification for acute inhalation toxicity has not been included in the GB MCL list.

[1] Meeting of the Competent Authorities for REACH and CLP

[2] Following a request from the European Commission on 12 May 2020, the Executive Director of ECHA requested RAC to prepare an opinion in relation to the acute inhalation toxicity of EGBE (pursuant to Article 77(3)(c) of Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals).

4. Justification that action is needed

The current GB MCL list entry for 2-butoxyethanol contains a minimum classification for acute inhalation toxicity (i.e., Acute Tox. 4*, H332). New information is available which suggests a more severe classification for acute inhalation toxicity may be warranted; the Agency has prepared this MCL report under Article 37A of GB CLP to assess the available data and decide whether the GB MCL needs to be updated.

5. Identified uses

According to the EU CLH report (ECHA 2017), 2-butoxyethanol, referred to as EGBE (ethylene glycol monobutyl ether) throughout this report, is part of the group of glycol ethers which are mainly used as solvents. Industrial uses include the manufacture of paints and surface coatings, detergents and surface cleaners, dyes, and inks. Further common uses include as an intermediate for 2-butoxyethanol acetate synthesis (including captive use) and as a cleaning agent. Additional minor uses for EGBE include in textile manufacture, and in the paper and rubber/oil industries.

6. Data sources

The study data used in this report was sourced from the initial CLH dossier by Germany (ECHA 2017), those submitted during the consultation of the initial report (ECHA 2018), the original RAC opinion (ECHA, 2018), those submitted during the consultation of the Article 77(3)(c) request and the RAC reassessment (ECHA, 2020).

Additional searches of UK and EU registration data were carried out. In addition, a literature search (Google Scholar, Pubmed) was carried out, covering publications produced over the time period of 01/2020 to 02/2024. The aim of this was to identify any new information published after the RAC re-assessment (2020). Search criteria used the relevant substance terms (e.g. ‘2-butoxyethanol’, ‘EGBE’, ‘ethylene glycol ether’), in combination with the terms ‘inhalation’ and ‘inhalation toxicity’.

7. Physicochemical properties

Table 7: Summary of physicochemical properties (adapted from ECHA, 2017)

Property	Value	Reference	Comment (e.g., measured or estimated)
Physical state at 20°C and 101,3 kPa	colourless liquid 1: Mild, ether-like odor. 2: Slight, rancid odor. 3: Weak, pleasant odor.	1. U.S. Department of Health & Human Services (2001) 2. Ashford (1994) 3. Gerhartz (1985)	
Melting/freezing point	-74.8 °C; 1 atm	Lide (1991) Lewis (1999) US National Library of Medicine (2008)	
Boiling point	170.2 °C; 1 atm	Riddick et al. (1986) Value cited is referenced to 6 original sources: Cretcher and Hightower (1924) Doolittle (1935) Newman et al. (1949) Scatchard and Satkiewicz (1964) Schneider (1959) Tallman (1934)	
Relative density	900 kg/m³, 20 °C	BASF AG (1992)	Measured
Vapour pressure	0.8 hPa, 20 °C	Merck KGaA (1996) Merck KGaA (2008)	
Surface tension	65.03 mN/m, 20 °C, 2 g/l	Binks (2005)	
Water solubility	miscible	BASF AG (1988)	Measured
Partition coefficient n-octanol/water	0.81, 25 °C	BASF AG (1987)	Measured
Flash point	61 °C	CHEMSAFE (2012)	Closed cup

Property	Value	Reference	Comment (e.g., measured or estimated)
Flammability	non flammable	BAM (2013)	Flammability upon ignition (solids, gases): Testing can be waived, substance is a liquid. Flammability in contact with water: The classification procedure needs not to be applied because the substance does not contain metals or metalloids. Pyrophoric properties: The classification procedure needs not to be applied because the substance is known to be stable into contact with air at room temperature for prolonged periods of time (days)
Explosive properties	no explosive properties	BAM (2013)	The classification procedure needs not to be applied because there are no chemical groups associated with explosive properties present in the molecule.
Self-ignition temperature	240 °C	CHEMSAFE (2012)	DIN 51 794
Oxidising properties	No oxidising properties	BAM (2013)	The classification procedure needs not to be applied because the organic substance contains oxygen atoms which are chemically bonded only to carbon or hydrogen.
Granulometry	/	/	/
Stability in organic solvents and identity of relevant degradation products	/	/	Based on existing data and the known properties of this substance, the stability of the substance in organic solvents is not considered critical. According to Annex IX, item 7.1.6 of the Reach Regulation, testing for stability is therefore not required.
Dissociation constant	pKa = 15, 20 °C	Karickoff (2007)	Measured
Viscosity	3.642 mm²/s (static), 20 °C 3.28 mPas	BP Chemicals Ltd (2002)	Measured

8. Evaluation of physical hazards

Not assessed.

9. Toxicokinetics (absorption, metabolism, distribution and elimination)

Table 8: Summary of toxicokinetic studies (adapted from the CLH report (ECHA 2017)).

Method	Results	Remarks	Reference
Animal inhalation data			
Toxicokinetics inhalation study (non-guideline, non-GLP) Fischer 344 (Rat) Male Nose only exposure, vapour Doses: 0, 0.024, 0.24, 2.18 mg/L (equivalent to 0, 5, 50, 450 ppm) Exposure duration: 6 hours Determination of fractional uptake of inhaled 2-butoxyethanol and body burden of the compound at the end of the exposure by respiratory measurements in 5 animals. After termination, entire carcasses were digested and 14C was used as a measure of the body burden. Determination of paths of excretion of 2-butoxyethanol equivalents and major metabolites carried out in a group of 4 rats. Collection of urine, faeces and exhaled breath for 66 h following exposure. After termination, entire carcasses were digested and 14C was used as a measure of the body burden. Determination of blood 14C and blood metabolite concentration at various time points and up to 24 h following exposure start in a 3.	**Absorption** No differences in respiratory rate and tidal volume, compared to unexposed rats. Amount of 2-butoxyethanol inhaled was proportional to exposure in the 5 and 50 ppm groups, less than proportional amount was inhaled at 450 ppm due to a lower minute volume. **Distribution** Majority of 2-butoxyethanol equivalent in the plasma. First 2h of exposure- 20% of blood 14C associated with blood cell fraction. Later time points had declining proportions of 14C in the cellular fraction (undetectable after exposure) Bound 14C metabolites were greater after exposure, compared to during exposure. **Metabolism** Major metabolite identified was butoxyacetic acid (BAA). Minor metabolites identified butoxyethanol glucuronide (BEG), ethylene glycol (EG). 2 further unidentifiable metabolites. **Excretion (via urine)** Majority of 14C eliminated via urine. < 7 % of the parent compound was exhaled following the exposure.	2-butoxyethanol (CAS-No.: 111-76-2) (purity: 99 %)	Sabourin *et al.* (1992)

Method	Results	Remarks	Reference
group of 30 rats (5 ppm exposure concentration only). Per time point, 3 rats were euthanized and bled by cardiac puncture. Blood samples were analysed for haematocrit (HCT), 14C associated with whole blood, 14C associated with red blood cells in plasma. Analysis of plasma metabolites.	10-20 % of (14C) 2-butoxyethanol equivalent remained in the carcass up to 66 h post exposure. 7 % of excreted 14C was in the form of $14CO_2$. More than 88 % of the total 14C was excreted in urine during the first 41 h. BAA was the major metabolite in urine, with EG and BEG found in lesser amounts. With increasing exposure concentration, proportion of unidentified minor metabolites increased. - At 5 ppm: 60 % of the urinary 14C was excreted during exposure - At 450 ppm: 10 % of the urinary 14C was excreted during exposure Metabolism to Glucuronide conjugate of 2-butoxyethanol (BEG) was favoured during the exposure, and metabolism to BAA and ethylene Glycol (EG) favoured post exposure.		
Toxicokinetic inhalation study (non-guideline, non-GLP) Sprague Dawley (Rat) Male (16/group) Whole body exposure, vapour Doses/conc.: 0, 0.096 mg/L and 0.48 mg/L (equivalent to 0, 20 ppm or 100 ppm) Exposure duration: 1, 2, 3, 4, 6, 8, 10 or 12 days Urine collection in 24 h intervals. Sacrifice, tissue samples: blood, muscle and liver (analysis of 2-butoxyethanol and BAA content).	No clinical signs of toxicity. 2-butoxyethanol concentration rapidly increased during the first three days and continue to increase slower during the remaining days of exposure. Average tissue concentrations of 2-butoxyethanol and BAA following 20 ppm exposure: blood: 10 – 20 µmol/l; liver: 10 µmol/l; muscle: 10 µmol/l; testis: 5 µmol/l: BAA concentration: blood: 30 – 40 µmol/l, liver: 15 – 20 µmol/l; muscle: 10 µmol/l; testis: 10 µmol/l. Following a 100 ppm exposure the tissue concentrations were approximately 5 times higher in blood,	2-butoxyethanol (CAS: 111-76-2) (purity: 99 %)	Johanson (1994)

Method	Results	Remarks	Reference
	3.5 and 3.6 times higher in muscle and testis, respectively, and 7.5 higher in liver. Estimated blood clearance of 2-butoxyethanol: to 2.6 ± 1.3 l/h/kg bw (not depending on dose administered). The urinary excretion of BAA averaged 0.2 mmol/day in the 20 ppm group and 1.03 mmol/day in the 100 ppm group. This corresponds to 64 % of the calculated respiratory uptake. The renal clearance was 0.53 l/h/kg.		
Toxicokinetic inhalation study (non-guideline, non-GLP) Fischer 344 (Rat) Male and female Whole body exposure, vapour Exposure doses/conc.: 0, 0.15 mg/L, 0.30 mg/L and 0.60 mg/L (equivalent to 0, 31.2, 62.5 or 125 ppm). Exposure duration: 6h/day, 5 days/week, 104 weeks Post exposure collection of blood: samples were collected after 1 day, 2 weeks and 3, 6, 12 and 18 months of exposure for 2-butoxyethanol and BAA determination. Post exposure collection of urine: samples were collected after 2 weeks and 3, 6, 12 and 18 months of exposure.	2-butoxyethanol blood concentrations rapidly dropped after exposure. Elimination half-time ($t_{1/2}$) for 2-butoxyethanol after 1 day of exposure: < 10 min, not dependent on dose level. Elimination of 2-butoxyethanol from blood seems to follow linear kinetics (mice faster than rats; male rats faster than female rats probably due to higher volume of distribution). Slower elimination rate ($t_{1/2}$) for 2-butoxyethanol after longer exposure. Identified metabolite: BAA BAA elimination from blood following saturable, non-linear kinetics. BAA was not rapidly cleared from the systemic circulation. BAA concentrations in the blood did not start to decline until 20 to 80 min post exposure (non-linear). Rate of BAA production reflects the 2-butoxyethanol elimination (mice faster than rats; male rats faster than female rats; higher blood concentrations in female rats; excretion of a lower amount of BAA in females, not depending on dose).	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %)	Dill *et al*. (1998)

Method	Results	Remarks	Reference
	Excretion rate of BAA tended to decrease with exposure time.		
Toxicokinetic inhalation study (non-guideline, non-GLP) B6C3F1 (Mouse) Female Whole body exposure, vapour Exposure dose/conc.: 1.2 mg/L (250 ppm) Exposure duration: 6 hours Mice (4 per time point) were terminated at 5 minutes, 24 and 48 hours post exposure. Whole body autoradiography of one animal for each time point, analysis of the free and bound radioactivity of the stomach and contents of the other 3 animals.	Whole body autoradiography <u>5 min after exposure:</u> - high level of radioactivity without showing preferential labelling in any tissue or organ - highest concentrations in liver, blood and nasal passages - high concentrations on the skin and fur near the hindquarters - lower concentrations in glandular mucosa of the stomach - no radiolabelling in the forestomach <u>24 hours after exposure:</u> - highest concentrations in liver and buccal cavity - high concentrations in mucosa of the caecum and forestomach mucosa, lower gastro-intestinal tract mucosa and oesophagus - conspicuously low, background levels in glandular stomach - high concentrations on skin and fur on the back and near hind quarters - lower level of labelling in salivary glands, thymus, kidney medulla, adrenal and spleen. - background labelling in the rest of the internal organs. <u>48 hours after exposure:</u> - high levels of radiolabelling in buccal cavity, oesophagus, forestomach, liver and mucosa of lower gastro-intestinal tract - high concentrations on skin and fur near the hind quarters - low levels in the duodenum, glandular stomach and remainder of internal organs Stomach and contents: - greater level of radioactivity due to 2-butoxyethanol in stomach and its contents immediately after exposure, than at	2-butoxyethanol (CAS: 111-76-2) (purity: 97.6 %)	Green *et al*. (2000)

Method	Results	Remarks	Reference
	later time points - at 24 and 48 h: more of 80 % of the radioactivity present in the stomach tissues covalently bound to protein - no difference between the glandular and forestomach. High radioactive concentrations on fur and skin, buccal cavity, oesophagus and stomach contents suggested to be due to grooming (during and post exposure) and mucous removal (muco-ciliary clearance) through the nasopharynx (during exposure). Retention of radioactivity in forestomach mucosa indicates that forestomach is a target organ following an inhalation exposure to 2-butoxyethanol.		
Toxicokinetic inhalation study (non-guideline, non-GLP) B6C3F1 (Mouse) Male and female Either whole body exposure or nose only exposure, vapour Exposure dose/conc.: 1.2 mg/L (250 ppm) Exposure duration: 6 hours After exposure, 5 mice were killed and immersed in hot water to collect 2-butoxyethanol deposited on the fur. Groups of 5 mice were killed immediately after inhalation exposure for blood analysis. Two groups of 5 mice were subjected to an 18 h urine collection after the inhalation exposures.	<u>Fur analyses</u>: - Average of 205 µg of 2-butoxyethanol on fur of mice exposed whole-body - Average of 170 µg of 2-butoxyethanol on fur of mice exposed nose-only - After corrections: 25 % more 2-butoxyethanol on the fur after whole body exposure, than after nose-only exposure. <u>Blood analyses:</u> - Mean 2-butoxyethanol concentrations: 3.0 and 3.9 mg/l for whole-body exposure and nose-only exposure, respectively. - Mean BAA concentrations: 235 and 390 mg/l for whole-body exposure and nose-only exposure, respectively. <u>Urine analyses:</u> - Low levels (about 68 µg) of 2-butoxyethanol 18 h after exposure to either route - Concentration supposed to come from the fur; 2-butoxyethanol is not expected to be excreted in the urine unconjugated.	2-butoxyethanol (CAS: 111-76-2) (purity unknown)	Poet *et al.* (2003)

Method	Results	Remarks	Reference
	- High free BAA levels (about 2020 µg and 1780 µg for whole-body exposure and nose-only exposure, respectively)		
Toxicokinetic inhalation study (non-guideline, non-GLP) B6C3F1 (Mouse) Male and female Whole body exposure, vapour Exposure dose/conc.: 0.3, 0.6, 1.2 mg/L (equivalent to 62.5, 125 and 250 ppm) Exposure duration: 6h/day, 5 days/week, 104 weeks Post exposure collection of blood samples were collected after 1 day, 2 weeks and 3, 6, 12 and 18 months of exposure for 2-butoxyethanol and BAA determination. Post exposure collection of urine samples after 2 weeks and 3, 6, 12 and 18 months of exposure. Before the core study started, a separate set of mice was moved into the control chamber and designated as the “aged (naïve)” mice. At 18 months into the chronic study, these mice (about 19 months old) were moved to the 125 ppm exposure chamber and exposed for 3 weeks. Blood collection after 1 day and 3 weeks of exposure at post exposure time points of 10, 20, 40, 80, 180, 360, 720 and 1440 min. Post exposure urine collection for 16 h after 2 weeks of exposure.	2-butoxyethanol blood concentrations rapidly dropped after exposure. Elimination half-time ($t_{1/2}$) for 2-butoxyethanol after 1 day of exposure: < 5 min, not dependent on dose level. Elimination of 2-butoxyethanol from blood seems to follow linear kinetics (mice faster than rats). Values of $t_{1/2}$ were significantly lower in mice at both exposure concentrations. Slower elimination rate ($t_{1/2}$) for 2-butoxyethanol after longer exposure. The kinetic parameters were not significantly different between male and female mice. BAA elimination from blood following saturable, non-linear kinetics. BAA was not rapidly cleared from the systemic circulation. BAA concentrations in the blood did not start to decline until 40 min post exposure (non-linear). Excretion rate of BAA tended to decrease with exposure time (mice faster than rats; no differences between males and females, but time-dependent changes not comparable between sexes). <u>Elimination of 2-butoxyethanol and BAA in aged mice:</u> - 2-butoxyethanol rapidly cleared from systemic circulation - kinetic parameters not different from those of young mice. - Age differences in elimination rate: slower terminal elimination phase in aged mice - no sex difference in elimination kinetic	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %)	Dill *et al.* (1998)

Method	Results	Remarks	Reference
	- blood concentration of BAA after 1 day of exposure 10x lower compared to young animals, $t_{1/2}$ higher in old mice - age-related difference disappeared after 3 weeks of exposure		
Human toxicokinetic data- inhalation			
Inhalation study - 4 human volunteers - Exposure via inhalation (whole body) - Exposure dose/conc.: 25 ppm (0.85 mmol/m3) - Exposure duration: 10 min Collection of exhaled air 1 min before and directly after exposure. Same people were also submitted to inhalation of 9 other substances in the same test conditions.	Mean respiratory rate for each solvent: 12.1 - 14 min^{-1}. Mean tidal volume for each solvent: 470 - 530 mL No differences among tested solvents. Conclusions: wash in/ wash out behaviour cannot completely explain actual respiratory behaviour of the tested solvents.	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Kumagai *et al.* (1999)
Inhalation study - 7 male human volunteers - Exposure via inhalation - Exposure dose/conc.: 20 ppm 2-butoxyethanol (0.85 mmol/m3) - Exposure duration: 2 h during light physical exercise (50W) Collection of blood samples during the exposure period and 3h afterwards for determination of 2- butoxyethanol concentrations. Collection of urine for a period of 24h. First sample collected immediately before the volunteer entered the exposure chamber and thereafter sampling at 2 h intervals for 6 hours for determination of 2- butoxyethanol and BAA concentrations.	No signs of adverse effects. Rapid increase in 2-butoxyethanol blood concentrations, reaching a plateau within 1-2 h. Rapid biphasic decay after exposure (semi-logarithmic plot). No detection of 2-butoxyethanol after 2- 4 h after exposure Average $T_{1/2}$ of 2-butoxyethanol: 40 min. Average plateau level in blood: 7.4 µmol/l. Average blood clearance: 1.2 L/min. Average steady-state volume of distribution: 54 L. Total amount of 2-butoxyethanol excreted via urine: less than 0.03 % of total uptake. $T_{1/2}$ of 2-butoxyethanol in urine: 1.36 h. Max. BAA concentration in urine: 5-12 h after start of exposure. Max. elimination: 2-10 h after start of exposure (great interindividual	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Johanson *et al.* (1986)

Method	Results	Remarks	Reference
	variations). $T_{1/2}$ for BAA in urine: 5.77 h		
Inhalation study - 5 human volunteers - Exposure via inhalation - Exposure dose/conc.: 20 ppm (0.85 mmol/m3) - Exposure duration: 2 h during light physical exercise (50W) Collection of venous blood for determination of BAA levels before and immediately after exposure and also at 4 and 6 h after exposure start. Collection of urine every 2 h for determination of BAA levels.	BAA in blood after 2 h of exposure. Average max. concentration of BAA (45 µM) after 2-4 h. Thereafter, decrease in BAA blood levels; average $T_{1/2}$: 4.3 h. Similar time profile in blood and urine, where the maximum occurred at about 5 h and $T_{1/2}$ was estimated to be 4 h. Average clearance of BAA: 23-39 ml/min (~1/3 of the glomerular filtration rate of about 125 mL/min). Lowe pKa of BAA: 3.5. Average Vd of BAA: 15 L. <u>Conclusions:</u> Low renal clearance due to binding of BAA to blood proteins and absence or low efficiency in tubular secretion. Low pKa of BAA indicates that more of 99 % of BAA present in urine is present in ionised form and is not available for tubular re-absorption at normal urine pH. Vd of BAA is approximately equal to the volume of extracellular water.	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Johanson and Johnsson (1991)
Inhalation studies, toxicokinetics - 4 human volunteers (2 males, 2 females) - Exposure via inhalation (whole body) - Exposure dose/conc.: 50ppm - Exposure duration: 2 h. Urine collections: after 0, 4, 6, 8, 10, 12, 22, 26 30, 24 h. Determination of creatinine, and free and total BAA levels. Blood collections: at 0, 0.5, 1, 1.5 and 2h (end of exposure), then every 20 min for a further 2 h. Determination of 2-butoxyethanol.	<u>Urine analysis:</u> Peak excretion 6-12 h post exposure. Mean half-life: 4h. Conjugation variable between individuals but does not slow elimination. <u>Haematology:</u> Mean peak blood concentration of 2-butoxyethanol: 7µM. Mean half-life: 56min. BAA: peaked 20 min after exposure (at average of 35µM). Mean half-life: 13 mins. <u>Breath measurements:</u> Maximum value only 12x LOD, so not deemed a reliable technique to	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Jones and Cocker (2003)

Method	Results	Remarks	Reference
Collection of breath samples: at 0, and 2h, then every 10-15 min for further 2 h.	quantify exposure.		
Inhalation studies, toxicokinetics <u>Experiment 1:</u> - 3 human volunteers (2 males, 1 female) - Exposure via inhalation (7900 L capacity with air drawn through at 1300L/ min) - Exposure dose/conc.: 200 ppm - Exposure duration: 2x 4 h, separated by 30 min <u>Experiment 2:</u> - 4 human volunteers (2 males (one also common in experiment 1), 2 females - Exposure via inhalation - Exposure dose/conc.: 100 ppm - Exposure duration: 2x 4 h, separated by 30 min Collection of urine (24 h samples, first collection at the end of the exposure day). Erythrocyte fragility test, blood pressure and pulse-rate were determined at the exposure day (3 measures: before, during exposure pause and after exposure). For erythrocyte fragility test, another measure was performed during exposure.	<u>Experiment 1:</u> One male and the only female excreted considerable amount of BAA within 4 h following exposure. The other male excreted only trace amount of BAA within the same period. Largest amount excreted by the female. <u>Experiment 2:</u> Urinary excretion of BAA. No other measured parameters changed significantly. Even one subject who had not excreted significant quantities of the metabolite after the 200 ppm exposure (experiment 1), did eliminate 75.5 mg BAA within 24 h. Urinary BAA levels of the other subjects similar to that found after the 200 ppm exposure (experiment 1). Female subjects generally experienced greater distress than males. <u>Haematology:</u> No adverse effects seen at either exposure concentration.	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Dow Chemical Company (1955)
Incidental, occupational exposure of workers of a beverage packing production - 31 male workers - Age 22–45 - Employed for 1–6 years - Low levels of airborne 2-butoxyethanol (~ 2.91 mg/m3 or 0.27 ppm) - Co-exposure to methyl ethyl ketone - Use of an unexposed control	No differences in RBC counts, Hb concentration, mean cell volume (MCV), mean corpuscular haemoglobin (MCH), haptoglobin and reticulocyte count, between exposed and control workers. Significant decrease in HCT (3.3 %). Significant increase in MCH concentration (MCHC; 2.1 %). Both values are within respective normal clinical ranges.	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown, incidental, occupational exposure)	Haufroid et al. (1997)

Method	Results	Remarks	Reference
group			
Human exposure study (worker biomonitoring) - 48 workers - Inhalative exposure (unintentional, occupational, incidental) - End shift urine measurements of free and total BAA	Urine: - No linear correlation between free and total BAA - Conjugation is an activated pathway that is triggered at urinary levels of 30 - 50mmol BAA/mol creatinine - Above this level: low ratio - Below this level: only some or no conjugation - Other data: conjugation has no effect on elimination rate	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Jones and Cocker (2003)

9.1 Short summary and overall relevance of the provided toxicokinetic information

To assess the toxicokinetic properties of EGBE after inhalation, six animal studies and seven human studies were evaluated.

In a study on the distribution of EGBE over time in mice (Green *et al.,* 2000), the substance was mainly distributed to the liver, blood, buccal cavity and the forestomach. This was indicated by the highest detected concentrations of EGBE and its metabolites in these areas.

Inhaled EGBE was mainly metabolised to butoxyacetic acid (BAA), ethylene glycol (EG) and butoxyethanol glucuronide (BEG). Increased doses of EGBE led to increased formation of BEG, compared to BAA and EG. BAA and EG form via a saturable mechanism.

Excretion of EGBE was shown to be rapid and occurred mainly via the urine. A small proportion was eliminated as CO_2 in expired air (< 10%). The half-life of EGBE in the blood was determined to be around 10 minutes in rats and 5 minutes in mice, independent of the exposure concentration. Elimination of EGBE follows linear kinetics, whereas BAA followed saturable, non-linear mechanisms. Repeat doses of EGBE resulted in decreased BAA elimination, with a slower elimination rate with prolonged exposure. Some species-specific differences in the rate of elimination were observed, with mice eliminating EGBE twice as fast as rats. Other differences included age-related findings in mice after short (24h) inhalative exposure, with older mice having a 10-fold lower BAA blood concentration compared to younger mice. However, this age-related difference was not observed following consistent exposure. In rats, the elimination of BAA varied by sex, with females eliminating BAA slower than males; this may be attributed to differences in renal excretion. No sex-related findings were observed in mice.

Human exposure studies of EGBE inhalation suggest that a ‘wash-in, wash-out’ mechanism of the respiratory tract is observed; this involves the hydrophilic substance becoming adsorbed to the respiratory tract during inhalation, and desorbed during exhalation. This leads to a decrease in the uptake of the substance. This is supported by animal experiments which show a similar pattern of rapid uptake of EGBE, peaking in plasma concentration at 2 hours, followed by decay. The half-life of EGBE was higher in humans than other animals (40 minutes). The main metabolite was identified as BAA, as seen in animal studies. As also seen in animal studies, excretion of EGBE and BAA was rapid and primarily via the urine.

10. Evaluation of health hazards

10.1 Acute toxicity – oral route

Not assessed.

10.2 Acute toxicity – dermal route

Not assessed.

10.3 Acute toxicity – inhalation route

Table 9: Summary of animal studies on acute inhalation toxicity (adapted from the RAC reassessment (ECHA, 2020))

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure, clinical signs/effects	LC_{50}	Reference
LC_{50} test, similar to OECD TG 403	Rat (strain not given), 6 females/group, and groups of older rats: 13 males/group, 23 females/group	2-butoxyethanol (CAS: 111-76-2), vapour (passing air at 2.5 L per minute through a fritted glass disc immersed in 50 mL of the liquid held at room temperature), no further details	800 ppm, 8h: 3/6 females 800 ppm, 4h: 0/6 females 500 ppm, 8h: 0/6 females 500 ppm, 4h: 1/6 females 375 ppm, 7h: 11/13 males 375 ppm, 7h: 23/23 females	Young female rats: 800 ppm, 8h (corresponding to 1008ppm/4h = 4.92 mg/L) Older male and female rats: 375 ppm, 7h (corresponding to 452 ppm/4h = 2.21 mg/L)	Carpenter *et al.* (1956) and Mellon Institute of Industrial Research (1952)
LC_{50} test, similar to OECD TG 403	Rat, F344, 6/sex/dose	2-butoxyethanol, purity 99.4 %, vapour	867, 523 or 202 ppm, 4h, whole body exposure, 14d post exposure observation period Mortality: 867 ppm, m+f: 6/6 on Day 2 523 ppm: m: 2/6, f: 3/6 during 14d post exposure period 202 ppm, m+f: 0/6 Necropsy deceased animals: enlarged and discoloured kidneys, urinary bladder	523 ppm = 2.56 mg/L Calculated 486 ppm = 2.37 mg/L (males) 450 ppm = 2.2 mg/L (females)	Bushy Run Research Center (1980a)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure, clinical signs/effects**	**LC_{50}**	**Reference**
			filled with red stained urine		
LC_{50} test, similar to OECD TG 403 (validation study, ring study)	Rat, Wistar, 3/sex/group	2-butoxyethanol (CAS: 111-76-2), purity 99 %, saturated vapour	617 ppm (3 mg/L) for 7h, 3h or 1h, whole body exposure, measurements in the exposure chamber: 750-910 ppm Mortality: 7h: m:1/3, f: 3/3; 3h: m: 0/3, f: 1/3; 1h: m/f: 0/3 Clinical signs: Lethargy, necrosis of the tail and haemolysis	617 ppm, 7h (corresponding to 743 ppm/4h = 3.63 mg/L/4h	Shell Chemicals (1982)
Inhalation hazard test, OECD TG 403, 1981 (interlaboratory trial, ring test data)	Rat, Sprague Dawley (Caw/Ico/Wiga (SPF); Wistar Bor: WISW (SPFCpb); Wistar (SPF); Wistar Alpk (AP); Wistar SHELL (SPF); Wistar Colworth; colony; 5/sex/group	2-butoxyethanol (CAS: 111-76-2), purity 99 %, saturated vapour	Nominal concentration: 3.1 to 4.1 mg/L (mean: 3.3-3.7 mg/L); estimated concentration 4.9 mg/L; head/nose exposure (1 lab), whole body exposure (5 lab: animals sat in cages in chamber or in tubes)	The 0-lethality time (LT_0, for which at least one death was found) was 3h for 5 laboratories and 1h for 1 laboratory.	Klimisch *et al.* (1988)
LC_{50} test, similar to OECD TG 403	Rat, 4/sex/group	2-butoxyethanol (CAS: 111-76-2), purity commercial grade, aerosol	2400 ppm (13 mg/L) for 5h, whole body exposure Clinical signs: comatose state, haematuria	LC_{100} = 2400 ppm, 5h (corresponding to 2585 ppm, 4h =	Gage (1970)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure, clinical signs/effects	LC_{50}	Reference
			Blood: Hb concentration 35 to 50 % of the normal Mortality: m/f: 4/4 on Day 2	12.62 mg/L/4h)	
Inhalation hazard test (IHT) according to Smyth (1962)	Rat, Sprague Dawley 6-12 males/females	2-butoxyethanol (CAS: 111-76-2), unknown purity, unknown form No further details given.	Mortality: 3h, 2.25 mg/L: 0/12 7h, 4.26 mg/L: 2/6. Clinical findings: Eyelid closure, slight salivation, accelerated respiration, haemorrhagic urine, apathy, crouch position, unstable gait, scrubby, contaminated fur, anaemic ears. Pathology findings in animals that died during the test period: *Heart*: acute dilatation on the right side, sallow left heart ventricle. *Lungs*: moderate acute exhalation. *Liver*: clay-grey tone. *Stomach*: bloody ulcerations in the area of the glandular stomach. *Intestine*: hematinic contents. Surviving animals were without findings	LC_{50}: > 4.6 mg/L/4h [NB: This was indicated to be 119% of the saturated vapour concentration; SVC]	BASF, 1979
IHT according to Smyth (1962)	Rat, species not specified 3 males/females	2-butoxyethanol (CAS: 111-76-2), no purity information	Mortality: 3h, 1.44 mg/L: 0/12 8h, 4.25 mg/L: 6/6	LC_{50}: 1.1-5.3 mg/L/4h	BASF, 1968

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure, clinical signs/effects**	**LC_{50}**	**Reference**
		provided, vapour			
LC_{50}-Test, no guideline followed An old acute study that pre-dates guidelines. Principles of current guideline methods followed, with more doses examined, increasing statistical precision of result. Some information on study protocols missing from publication. Missing statistical analysis.	Mouse, strain and number of animal used not given The study also assessed Wistar rats (23/group, sex unspecified) and dogs (species unspecified, 2/group). Details of the additional rat/dog studies were included as part of the original CLH report, but the study protocols and data are lacking. The inclusion of the data here is to represent additional supporting information on haematological end points, rather than to be used as a solely reliable source.	2-butoxyethanol (CAS: 111-76-2), purity commercial grade, vapour	**Mice:** Specific dose levels and exposure durations were not available. Clinical signs in mice: Dyspnoea, haemglobulinurea, death were noted in the 4th week after exposure. Necropsy: findings in spleen, liver, lungs, and kidneys. **Rats:** 0, 135, 320 ppm for 7 hours/day, 5/days/week, 5 weeks Haematology: increased percentage of circulating immature granulocytes, decreased Hb concentrations and RBC counts, and increased reticulocyte counts. Reversed 3 weeks after discontinuing exposure **Dog:** 0 or 415 ppm, 7 hours/day, 5/days/week, 12 weeks. Haematology: decreased Hb concentration and RBC count with increased hypochromia, polychromatophilia, and microcytosis. Effects reversed 5 weeks post-exposure.	700 ppm, 7h (corresponding to 843 ppm/4h = 4.12 mg/L/4h) in mice No further LC_{50} values were available.	Werner *et al*. (1943) cited in Carpenter *et al*. (1956)
LC_{50} test, no guideline followed	Guinea pig, strain unspecified, adult	2-butoxyethanol (CAS: 111-76-2), “Substantially	1300 ppm for 7h, whole body exposure, 14d post exposure period	1300 ppm, 7h (corresponding to 1566 ppm/4h =	Mellon Institute of Industrial Research

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure, clinical signs/effects**	**LC_{50}**	**Reference**
		saturated vapour"		7.65 mg/L/4h)	(1943) cited in Tyler (1984)
LC_{50} test, similar to OECD TG 403; deviation in exposure time, only 1h was used	Guinea pig, Hartley strain (5 wk of age; 400- 500 g), 5/sex	2-butoxyethanol (CAS: 111-76-2), purity 99.87 %, vapour	633±14.2 ppm (males) and 691±37.6 ppm (females) for 1h, whole body exposure, 14d post exposure period	No mortalities > 633 ppm (males) > 691 ppm (females)	Dow Chemicals Company (1994) also referred to as Gingell *et al.* (1998)

<table>
<tr>
<td>Acute inhalation toxicity study/LC_{50}-test. No guideline, non-GLP

Three groups of animals, single dose exposure to 3 different samples, fourth control group

Experiment 1: all animals exposed for 7h, observed for 7 days post-exposure. Guinea pigs (4/8) and rabbits (4/4) sacrificed for pathological examination, dogs physically examined.

Experiment 2: 1 week post-exposure, dogs (2/2) and guinea pigs (4/8) re-used. Additional 4 guinea pigs and 8 rabbits not previously used added, exposed under same conditions</td>
<td>Dog, Beagle
2 male

Rabbit, albino
4 male

Guinea pig, species unknown
8 male</td>
<td>2-butoxyethanol

Three different purified samples used (Dowanol EB, BO-USA, BO-Europe), vapour

% Purity unknown</td>
<td>1 m^3 chamber under dynamic conditions, 27-27.5°C

Nominal vapour concentration of 400 ppm, 7h exposure duration, 7 day post-exposure observation.

No mortality or adverse effects in dogs or guinea pigs across both experiments.

Moribund state in rabbits 8-16h post exposure followed by death (experiment 1 and 2).

<table>
<tr><th>Sample</th><th>1st experiment</th><th>2nd experiment</th></tr>
<tr><td>Dowanol EB</td><td>3/4</td><td>3/4</td></tr>
<tr><td>BO-USA</td><td>1/4</td><td>0/4</td></tr>
<tr><td>BO-Europe</td><td>0/4</td><td>3/4</td></tr>
<tr><td>Total:</td><td>4/12</td><td>6/12</td></tr>
</table>
</td>
<td>> 2.36mg/L extrapolated to 4hrs exposure

(48% of SVC)

in all animals</td>
<td>Dow Chemicals Company (1974)</td>
</tr>
</table>

as experiment 1. Additional exposure to stress in the form of loud noise, observed 7-days post exposure. Guinea pigs and rabbits sacrificed for examination, dogs used in other studies. **Experiment 3**: repeat exposure of 400 ppm 7h/day for 5 days, to assess contradictory results.					

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure, clinical signs/effects**	**LC_{50}**	**Reference**
Acute inhalation toxicity/LC50-test. OECD TG 433 with deviations, GLP Single inhalation, nose only exposure Deviations included the use of 6 animals per sex Clinical condition, body weight, haematology (peripheral blood), urinalysis, organ weight and macroscopic examinations done. The mean achieved exposure level was 2.25 mg/L (=75% of the targeted concentration; 3 mg/L); this was considered the	Guinea pig, Dunkin Hartley 6/sex Aged 42-56 days on day of exposure	2-butoxyethanol (EGBE), purity 99.61%	Input dose: 3.0 mg/L, vapour Established mean exposure concentration: 2.25 mg/L ± 0.19 mg/L 4 hours Mortality in one animal, considered to be unrelated to treatment. No clinical, haematological or pathological findings.	≥ 2.25 mg/L/4h	Covance CRS Limited (2019) Data also cited in Kelsey et al. (2023)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure, clinical signs/effects	LC_{50}	Reference
maximum achievable stable vapour only concentration under the study conditions.					

Table 10: Summary of human data on acute inhalation toxicity (adapted from the CLH report (ECHA, 2017))

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Not described.	2- butoxyethanol (CAS: 111- 76-2), purity commercial grade, vapour	Exp. 1: Exposure of 2 men to 113 ppm (0.55 mg/L) for 4h, and one year later exposure of the same 2 men and one woman to 195 ppm (0.95 mg/L) for two 4h periods separated by a 30-min interval Exp. 2: Exposure of 2 men and 2 woman to 98 ppm (0.48 mg/L) for 8	Clinical signs: Irritation to the eyes (probably due to direct contact with the vapours), 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 the men No evidence of changes from pre-exposure values in erythrocyte fragility, blood pressure, pulse rate or urinary levels of glucose or albumin; urinary excretion of BAA (100-200 mg) with the next 24h with considerable individual variation Haematology: No adverse effects seen at either exposure concentration.	Carpenter *et al*. (1956)
Determination of pharmacokinetic	2- butoxyethanol (CAS: 111- 76-2),	Exposure of 4 male volunteers to 50 ppm (0.24 mg/L) for 2h in an open-system exposure	50 ppm: No consistent effects on the lungs (ventilation or breathing rate) or the heart	Johanson (1986)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
data	purity commercial grade, vapour	chamber	(electrocardiogram readings or heart rate	
Determination of the respiratory uptake	2- butoxyethanol (CAS: 111- 76-2), purity commercial grade, vapour Exposure of 7 male volunteers (age range 23-36, bw 75-80 kg, body length 178-187 cm) to 50 ppm (0.24 mg/L) for 2h v	Exposure of 7 male volunteers (age range 23-36, bw 75-80 kg, body length 178-187 cm) to 50 ppm (0.24 mg/L) for 2h vapour inhalation (through the mouth alone)	50 ppm: No overt signs of toxicity	Johanson and Boman (1991)

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

Saturated Vapour Concentration (SVC)

The concentration of vapour in inhalation toxicity studies can vary based on the temperature and the system used to generate the vapour. At a temperature of 20°C, the theoretical maximum concentration of EGBE vapour is equal to 3.9 mg/L. However, with an increase in temperature, the concentration also increases; at 25°C, the theoretical maximum would be approximately 5.6 mg/L. In accordance with OECD test guidelines, chamber temperatures should be maintained at 22 ± 3°C, i.e. within 20-25°C. RAC, in their reassessment (ECHA, 2020) suggested that this results in a saturated vapour concentration range of 3.9 – 5.6 mg/L, depending on the temperature (20-25°C). Maintenance of the ideal conditions used in inhalation toxicity studies is challenging, due to the dynamic nature of the testing system. For example, temperature changes between the vapour generation system and the exposure chamber, loss/deposition of test material, or the formation of mist as a result of condensation, may all result in a failure to meet and/or maintain the maximum theoretical concentration (Kelsey *et al.,* 2023). Some of the studies for the assessment of inhalation toxicity detailed the vapour generation systems and the temperature of the air inhaled by animals.

For volatile substances, the differentiation between vapour and mist is made on the basis of the saturated vapour concentration (SVC). An LC_{50} well below the SVC is considered according to the classification criteria for vapours, whereas an LC_{50} close to or above the SVC is classified according to the criteria for mists. When adjusting for Haber's law, the LC_{50} values reported in the animal studies (detailed below) were 2.21 – 4.92 mg/L/4h (rats), > 2.25 – 2.36 mg/L/4h (guinea pig), > 2.36 mg/L/4h (dog and rabbit) and 4.12 mg/L/4h (mouse). It is recognised that in most cases, the LC_{50} values had to be extrapolated to give the equivalent value for a 4 hour exposure to allow comparison to the classification criteria.

Animal studies

A number of animal studies were available for the assessment of acute inhalation toxicity. In addition to the studies available in the original CLH report, further studies were submitted during the public consultation on the CLH report, and during the public consultation on the Article 77(3)(c) request. These studies conducted in rats, mice, guinea pigs, dogs and rabbits are all considered by the Agency in this MCL report. For comparison with the CLP classification criteria, LC_{50} values were adjusted using Haber's law.

In rats, seven studies were available. Most studies were carried out prior to the introduction of OECD test guidelines and GLP, which results in varying exposure times outside of the 4 hours specified in the classification criteria. Across the rat studies, the LC_{50} values ranged from 2.21 – 5.3 mg/L/4h. Two studies were unable to calculate an LC_{50} value; Gage (1970) measured an LC_{100} of 12.62 mg/L/4h, and Klimisch *et al.* (1988) measured a 0-lethality time (LT0) of 3h and 1h for which at least one death was found. In most studies, the LC_{50} value for rats were below indicated saturated vapour concentrations.

In a study by BASF (1979), Sprague Dawley rats were exposed to EGBE at a concentration of 2.25 mg/L for 3 hours, or 4.26 mg/L for 7 hours. Mortality was observed in 2/6 animals in the higher concentration group, but not in the 2.25 mg/L group. The LC_{50} was calculated as > 4.6 mg/L/4h which is 119% of the saturated vapour concentration. In a second study by BASF (1968), rats were exposed to EGBE at concentrations of 1.44 mg/L for 3 hours or 4.25 mg/L for 8 hours. No mortality was observed in the 1.44 mg/L group, however, 6/6 animals in the higher concentration group died. The LC_{50} was calculated as between 1.1-5.3 mg/L/4h.

Across most studies, clinical signs observed in exposed rats included unstable gait, lethargy, laboured breathing and tail necrosis. Pathological and haematological findings included discoloured kidneys, haematuria and haemoglobinuria (Carpenter *et al.* 1956; Bushy Run Research Center 1980a; Shell Chemicals 1982; Gage 1970), with one study (Gage 1970) reporting a 50-65% reduction in haemoglobin levels.

In one rat study, intraspecies differences were observed (Carpenter *et al*. 1956). Females and older animals (LC_{50}: 2.21 mg/L/4h) were considerably more sensitive to EGBE compared to males and younger animals (4.92 mg/L/4h).

One mouse study by Werner *et al.* (1943) (cited by Carpenter *et al.,* 1956) calculated the LC_{50} as 4.12 mg/L/4h. Similar clinical signs of dyspnoea, haemoglobinuria and necropsy findings (no further details) within the kidneys were noted.

In dogs, one study was available for assessment (Dow Chemicals Company, 1974) (submitted during the consultation on the original CLH dossier). Two male Beagles were exposed to EGBE for 7h at a concentration of 1.96 mg/L, with the LC_{50} calculated by extrapolation as > 2.36 mg/L/4h. No mortality or clinical effects were reported, aside from increased salivation. No urinalysis was performed with the study. The concentration achieved was low and well below the SVC (48% of SVC).

In rabbits, one study by Dow Chemicals Company (1974) (submitted during the consultation on the original CLH dossier) exposed four males to 2 mg/L of EGBE for 7h, across two experiments. Three different samples of EGBE were used. In the first experiment, no adverse effects were observed during or directly after exposure. However, within 8-16h post exposure, 3/4 rabbits in one sample group (Dowanol EB) were found in a

moribund condition, and subsequently died. An additional rabbit died in another sample group (BO-USA). In the second experiment, which was carried out under the same conditions but with added stress (in the form of loud noise), similar signs of toxicity were seen. At this concentration, a 50% mortality rate was observed. Clinical signs included poor co-ordination and loss of equilibrium. Pathological examination of rabbits who died showed red discharge in the eyes and nose, kidney congestion, discoloured livers and haematuria. Surviving rabbits showed darkened or congested kidneys and mottled livers. The LC_{50} was calculated as 2.36 mg/L/4h, a concentration that was 48% below the SVC.

In guinea pigs, four studies were available for assessment. The majority of these studies were not performed to test guidelines or GLP.

In one study (Mellon Institute of Industrial Research, 1943, as cited by Tyler, 1984), the LC_{50} was reported as 7.65 mg/L/4h, with no reporting of adverse effects. Since the concentration of EGBE was above the saturated vapour pressure, the actual exposure was due to, in part, an aerosol of EGBE rather than vapour. As a result, as well as the lack of reported effects, this study is not considered as relevant for classification.

A second study (Dow Chemical Company, 1994; Gingell *et al.* 1998) used a lower concentration of approx. 3.1-3.4 mg/L for 1h of whole body exposure. No mortality or clinical effects were observed. However, as the exposure time was below 4 hours, an LC_{50} could not be set and this study is considered inconclusive for classification purposes.

In another study by Dow Chemical Company (1974), male guinea pigs were exposed to EGBE at a concentration of approximately 1.95 mg/L for 7 hours. No other concentrations were tested. No mortality or clinical effects were observed. No pathology was reported, as the study was not performed for the purposes of calculating an LC_{50}. The Article 77 (3)(c) request consultation indicated that the LC_{50} value for this study was > 2.36 mg/L/4h.

A guideline study (OECD TG 433) by Covance CRS Limited (2019) aimed to investigate acute inhalation toxicity in guinea pigs via single nose-only exposure, whilst utilising the highest technically attainable saturation concentration of EGBE vapour. Guinea pigs (6/sex) were exposed to EGBE for 4h, at a mean exposure concentration of 2.25 mg/L ± 0.19 mg/L, and a mean temperature of the air inhaled through the snout of 22.4 ± 0.4 degrees Celsius. The mean concentration was deemed acceptable as it was 75% of the planned concentration of 3 mg/L. The study authors considered this to be the highest technically achievable vapour only concentration, with no mists observed during the exposure period. No further explanation as to why this particular system yields the highest technically achievable concentration was provided. One animal was euthanised on day 5 for welfare reasons; clinical signs included impaired locomotion, body weight loss and a distended GI tract. These findings were considered to be unrelated to treatment. No other mortality or clinical signs were observed in the other animals. No treatment-related

changes in haematology, organ weight, or pathology were observed. The LC_{50} was determined to be ≥ 2.25 mg/L/4h.

Human data

In humans, three sets of data were available for the assessment of acute inhalation toxicity. No effects on the lungs, heart, or any overt signs of toxicity were observed when male volunteers were exposed to 0.24 mg/L for 2 hours (Johanson 1986; Johanson and Bowman 1991).

In a study by Carpenter *et al*. (1956), two men were exposed to 0.55 mg/L of EGBE for 4h. A year later, the same two men and an additional two women were exposed to 0.95 mg/L of EGBE for two 4 hour periods, separated by a 30 min interval. Some clinical signs observed included irritation of the eyes, nose and throat, an increase in nasal mucous discharge and headache. No changes in pre-exposure values of erythrocyte fragility, blood pressure, pulse rate or urinary levels of glucose and albumin were observed. Levels of butoxyacetic acid (BAA), the main metabolite of EGBE, in the urine were variable between individuals. In this study, females appeared to be more sensitive to adverse effects whilst excreting the largest amount of BAA. No adverse effects on haematology were observed at either exposure concentration.

The effects observed in Carpenter *et al.* (1956) showed clear local irritative effects on the eyes and respiratory tract at a lower concentration. The concentration (0.95 mg/L) was well below saturated vapour concentrations (3.9-5.6 mg/L, temp 20-25°C), meaning toxic effects may be more severe at higher concentrations.

Modes of Action

The toxic effects EGBE are suggested to be a result of its main metabolite, butoxyacetic acid (BAA). It has been established that BAA can cause rapid haemolysis after acute exposure, with the sensitivity of this effect varying between species (Ghanayem and Sullivan 1993). Recent reviews of the data by Boatman *et al.* (2014) suggest that BAA-mediated intravascular haemolysis is the primary toxic effect in rats and rabbits, as supported by mechanistic data. Rats, mice and rabbits are sensitive to haemolysis whereas humans and guinea pigs are resistant. As a result, it was suggested by industry that as humans and guinea pigs have comparable sensitivity to haemolysis, only the guinea pig data should be considered for classification.

Various acute human cases were referenced in the original CLH report, with RAC suggesting that BAA-haemolytic resistant species may have alternative mechanisms of action for acute toxicity. They noted that the primary toxic effect for humans in oral poisoning was likely to be metabolic acidosis, due to the high concentration of BAA in the

blood. No haemolysis was reported in these studies. Severe effects often included acidosis alongside CNS depression, with some patients reported to have breathing difficulties and pulmonary oedema. The estimated oral dose of EGBE that leads to severe acute poisoning in humans has been reported as a range of 500-1250 mg/kg bw, with the exception of one case at 4.5 g/kg bw.

In their reassessment, RAC suggested that the oral doses of EGBE which induced severe intoxication in humans were comparable to the median lethal oral dose in laboratory animals. Despite variation, they noted that the oral LD_{50} values for rats were within a range of 1480-2420 mg/kg bw, and within a similar order of magnitude as guinea pigs at 1200-1414 mg/kg bw. They noted that the similar oral LD_{50}s across the species indicated that the haemolytic action would have limited influence on the potency of acute toxicity when causing lethal acute poisoning. Based on the oral exposure data, they suggested that haemolysis is not the only mode of action, and that human susceptibility to acute toxicity of EGBE is similar to that in rats, mice and guinea pigs. The Agency notes that as the exposure route differs between inhalation and oral exposure, this may have an impact on the severity and type of effects observed in humans. However, it is agreed that the potential mechanisms of action leading to toxicity are likely to be similar, meaning that human relevance cannot be fully excluded. Despite differences in toxicokinetics and metabolism, RAC considered that there was no evidence of a major difference in sensitivity when comparing guinea pigs to rats, and that data from both should be considered in the classification assessment. The rat is usually the preferred species for assessing acute inhalation toxicity. When considering the study data for rats, there were clear adverse effects including mortality, haemolysis, and signs of ataxia. Rabbits displayed similar adverse effects, with the addition of more severe pathological findings in the liver and kidneys. In comparison, guinea pigs, which are suggested as a more suitable model for this case of human health assessment, a lack of mortality and adverse effects were observed in the available inhalation toxicity studies. The Agency notes that haemolysis is not the only potential mode of action, however no investigations into acidosis or alternate mechanisms of action were carried out in guinea pigs. Although it has been noted that rats and rabbits are particularly sensitive to haemolysis, data from these species should not be excluded and is appropriate for use in a weight of evidence assessment.

Discussion on SVC

In their assessment, RAC concluded that the LC_{50} values in three animal species: 2.2–4.92 mg/L/4h in rats (Carpenter *et al.,* 1956; Mellon Institute of Industrial Research, 1952; Bushy Run Research Center, 1980a; Shell Chemicals, 1982; BASF, 1979; BASF, 1968), 4.12 mg/L/4h in mice (Werner et al., 1943 cited by Carpenter *et al*.,1956), and 2.36 mg/L/4h in rabbits (Dow Chemical Company, 1974) provide sufficient evidence that EGBE

meets the criteria (LC_{50} in a range of 2-10 mg/L of air) given in the CLP Regulation, for classification of a substance present in air as a vapour, as Acute Toxicity Category 3 via the inhalation route.

The Agency notes that many of the studies available for assessment lack information on the systems used for the generation of vapours, as well as characterisation of the test atmosphere. The low volatility and low vapour pressure of EGBE mean that there is potential for the exposure of a mixture of vapour and mist, rather than pure vapour. The concentrations which approach the SVC are more likely to form this mixture, especially in the context of experimental variation. In addition, many of the studies pre-dated guidelines and took different approaches to atmosphere generation, without checks to ensure the absence of mists (with the exception of Gingell *et al.* 1998). The Agency is of the opinion that studies which approached the lower end of the SVC range are more likely to be vapour, and that the results observed in these studies are more informative for classification.

Kelsey *et al.* (2023) aimed to evaluate existing studies in order to calculate a maximum attainable vapour concentration in the absence of mist formation. The paper provides calculations on a theoretical measurement as well as a practical achievable concentration, which ranged between 2.07-4.57 mg/L at 295K (based on various parameters such as experimental error of vapour pressure, and enthalpy of evaporation). These calculations were then used to carry out trials prior to the main guideline animal study, which is described in Covance CRS Limited (2019). The intended target concentration was 3.0 mg/L, with actual measurements of 2.25 mg/L ± 0.19 mg/L. The authors argued that in the case of EGBE, this was the maximum practical achievable concentration. The theoretical concentrations assume ideal behaviour, but in practice, thermodynamic parameters and experimental conditions are subject to variation. The Agency recognises that maintaining dynamic test atmospheres is challenging and would lead to a difference between a theoretical versus a practical achievable concentration. However, other studies cannot be dismissed based on the notion that this is the maximum achievable SVC; other systems of exposure and atmosphere generation may have their own maximum SVC unique to the system.

10.3.2 Comparison with the GB CLP criteria

Fourteen animal studies and three human studies were available for the assessment of the acute inhalation toxicity of EGBE.

Under CLP, acute inhalation toxicity is assigned on the basis of adverse effects following an inhalation exposure of 4 hours. In particular, acute toxicity is characterised based on evident lethality, or the potential to cause lethality.

When adjusted using Haber's law, the LC_{50} values were 2.21 – 4.92 mg/L/4h (rats), 2.25 – 2.36 mg/L/4h (guinea pig), 2.36 mg/L/4h (dog and rabbit) and 4.12 mg/L/4h (mouse).

According to CLP Annex I, 3.1.2 to 3.1.3.4, the classification criteria for inhalation (vapour) of an LC_{50} >2 but ≤ 10.0 mg/l results in a classification of Acute Tox. 3.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

The Agency concludes that **2-butoxyethanol (EGBE) meets the criteria for classification as *Acute Tox. 3; H331 (Toxic if inhaled),* with the default ATE of 3.0 mg/L/4h (vapour).**

10.4 Specific target organ toxicity – single exposure (STOT SE)

Not assessed.

10.5 Skin corrosion/irritation

Not assessed.

10.6 Serious eye damage/eye irritation

Not assessed.

10.7 Respiratory sensitisation

Not assessed.

10.8 Skin sensitisation

Not assessed.

10.9 Specific target organ toxicity – repeated exposure (STOT RE)

Not assessed.

10.10 Germ cell mutagenicity

Not assessed.

10.11 Carcinogenicity

Not assessed.

10.12 Reproductive toxicity

Not assessed.

10.13 Aspiration hazard

Not assessed.

11. Evaluation of environmental hazards

Not assessed.

12. Evaluation of additional hazards

12.1 Hazardous to the ozone layer

Not assessed.

13. Additional labelling

No additional labelling is proposed.

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