Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

2-butoxyethanol; ethylene glycol monobutyl ether

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

CLH-O-0000001412-86-226/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

14 September 2018

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

International Chemical Identification:

2-butoxyethanol; ethylene glycol; monobutyl ether

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

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

Index Number: **603-014-00-0**

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Version number: 1.1 **Date: August 2017**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 2-BUTOXYETHANOL; ETHYLENE GLYCOL; MONOBUTYL ETHER

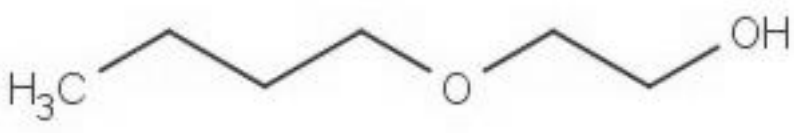
CONTENTS

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 2-BUTOXYETHANOL; ETHYLENE GLYCOL; MONOBUTYL ETHER

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 butyl glycol
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 (Annex VI Index number)	603-014-00-0
Molecular formula	$C_6H_{14}O_2$
Structural formula	
SMILES notation (if available)	OCCOCCCC
Molecular weight or molecular weight range	118.17 g/mol

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 CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
2-butoxyethanol	99.5		

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 CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
none				

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 CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
none					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: The current Annex VI entry and the proposed harmonised classification for 2-butoxyethanol

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	H332 H312 H302 H315 H319			
Dossier submitters proposal	603-014-00-0	2-butoxyethanol ethylene glycol monobutyl ether	203-905-0	111-76-2	**Retain:** Skin Irrit. 2 **Modify:** Acute Tox. 4 Acute Tox. 3 Acute Tox. 3 Eye Dam. 1 STOT RE 2	**Retain:** H315 H302 **Modify:** H311 H331 H318 H373 (blood)	**Add:** GHS05 GHS06 GHS08 Dgr **Delete:** GHS07 Dgr	**Retain:** H315 H302 **Modify:** H311 H331 H318 H373 (blood)		**Add:** inhalation: ATE = 3 mg/L dermal: ATE = 300 mg/kg bw oral: ATE = 500 mg/kg bw	
Resulting Annex VI entry if agreed by RAC and COM	603-014-00-0	2-butoxyethanol ethylene glycol monobutyl ether	203-905-0	111-76-2	Acute Tox. 4 Acute Tox. 3 Acute Tox. 3 Skin Irrit. 2 Eye Dam. 1 STOT RE 2	H332 H311 H302 H315 H318 H373 (blood)	GHS05 GHS06 GHS08 Dgr	H331 H311 H302 H315 H318 H373 (blood)		**Add:** inhalation: ATE = 3 mg/L dermal: ATE = 300 mg/kg bw oral: ATE = 500 mg/kg bw	

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

Hazard class	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	harmonised classification proposed	Yes
Acute toxicity via dermal route	harmonised classification proposed	Yes
Acute toxicity via inhalation route	harmonised classification proposed	Yes
Skin corrosion/irritation	harmonised classification proposed	Yes
Serious eye damage/eye irritation	harmonised classification proposed	Yes
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	harmonised classification proposed	Yes
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 PREVIOUS CLASSIFICATION AND LABELLING

The current acute toxicity classification for 2-butoxyethanol is based on Directive 67/548/EC and translates into a minimum classification of 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." according to the CLP Regulation. 2-butoxyethanol is further classified as Skin Irrit. 2 H315: "Causes skin irritation." and Eye Irrit. 2 with the hazard statement H319: "Causes serious eye irritation.".

Minimum classification for a category is indicated by the reference *.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[B.] Justification that action is needed at Community level is required.

Change in existing entry due to changes in the criteria
Change in existing entry due to new interpretation/evaluation of existing data

Further detail on need of action at Community level

2-butoxyethanol is manufactured and/or imported in the European Economic Area in 100,000 – 1,000,000 tonnes per year. The current acute toxicity classification of 2-butoxyethanol is a minimum classification according to Directive 67/548/EEC. For certain hazard classes, including acute toxicity and STOT repeated exposure (STOT RE), the classification according to the criteria in Directive 67/548/EEC does not correspond directly to the classification in a hazard class and category under the CLP Regulation. If new data or other information as specified in Part 1 of Annex I of the CLP Regulation is available that lead to classification in a more severe category compared to the minimum classification, as it is the case with 2-butoxyethanol, a classification in the more severe category must then be applied.

The re-evaluation of all available data on Acute Tox., Eye Irrit., Skin Irrit. and STOT RE resulted in a justified classification of this substance as Acute Tox. 4 (oral; H302), Acute Tox. 3 (inhalation, H331), Acute Tox. 3 (dermal, H311), Skin Irrit. 2 (H315), Eye Dam. 1 (H318) and STOT RE 2 (H373). The new classification according to CLP criteria substitutes the minimum classification, since it differs from it, and thus a proposal for harmonised classification is justified.

Re-evaluation of 2-butoxyethanol was triggered by an enforcement enquiry to the German CA based on a refusal of a manufacturer to classify 2-butoxyethanol appropriately according to the CLP Regulation. Hence, laying down the classification at EU level, and therefore submission of a CLH proposal for 2-butoxyethanol was deemed necessary by the German CA.

5 IDENTIFIED USES

The chemical 2-butoxyethanol belongs to the group of glycol ethers, which are mainly used as solvents. This substance has a wide range of uses as a solvent in paints and surface coatings, detergents and surface cleaners, inks or dyes. The use of 2-butoxyethanol in paint and lacquer industry represents ~ 58 % of the total volume used in EU (between 2001 and 2003). The two other main uses, intermediate for 2-butoxyethanol acetate synthesis (including captive use) and cleaning agent, represent respectively ~ 20 % and ~ 11 % of the total quantity of 2-butoxyethanol used. Information for other minor uses for 2-butoxyethanol is also available (e.g. paper industry, textile manufacture, rubber/oil industry). The sum of the other uses represents about 10 % of the total use of 2-butoxyethanol.

6 DATA SOURCES

A literature enquiry was performed and data were obtained from the registration dossiers.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

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.
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 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of toxicokinetic studies. The study documentation is organised regarding route of application (oral < inhalation < dermal < other routs), species (always: rats < mice < other species) and study duration (ascending), followed by studies in humans (oral < inhalation < dermal) and other studies, such as *in vitro* or computer modelling studies.

Method	Results	Remarks	Reference
Oral			
Metabolic and disposition study *In vivo* No TG followed No GLP compliance (study considered reliable with restrictions) **Fischer 344 rats** - Males (3/dose) - Exposure by gavage- - Exposure doses/conc.: [14C] 2-butoxyethanol at 10 and 125 mg/kg bw (aqueous solution) and an additional group dosed with [14C] 2-butoxyethanol at 10 mg/kg in a corn oil vehicle Collection of blood at 1, 3, 6, 12 and 24 h after dosing for determination of total 14C (plasma) and for analysis of 2-butoxyethanol and butoxyacetic acid (BAA; in whole blood). Collection of urine at intervals of 0-12 and 12-24 h after dosing to assess metabolic profile. Collection of faeces for 24 h. Collection of expired $14CO_2$ throughout the study.	125 mg/kg bw: - Haematological effects in 2 rats (the third rat was eliminated because of mis-dosing). - 37.7 and 70.3 % of the dose excreted in urine during the first 24 h (2 rats) - 7.6 and 8.5 % of the dose excreted as $14CO_2$ during the first 24 h (2 rats) - 65 % of the 14C in urine present as BAA during the first 12 h - 10 % of the 14C in urine present as glucoronidase-labile conjugate of 2-butoxyethanol 10 mg/kg bw: - 59 % of the dose excreted in urine during the first 24 h following administration (in either corn oil or in water) - 10 % of the dose excreted as $14CO_2$ in 24 h. - 40 % of the 14C in urine present as BAA during the first 12 h - 15 % of the 14C in urine present as glucoronidase-labile conjugate of 2-butoxyethanol Maximum radioactivity plasma concentration apparently just before first blood sample collection. Ethylene Glycol (EG) confirmed as metabolite of 2-butoxyethanol, but only present in small quantities.	2-butoxyethanol (CAS-No.: 111-76-2) (purity: 96.3 %)	Dow (1993)
Metabolic and disposition study No TG followed No GLP compliance (study considered reliable with restrictions) **Fischer 344 rats** - Males - Exposure by gavage - Exposure duration: single doses of radiolabeled 2-butoxyethanol - Exposure dose/conc.: 125	- Amount of $14CO_2$ exhaled in the first 48 hours after dosing : ~18 and 10 % of administered dose - Exhaled Volatiles accounted for ~ 2 % of administered dose - Faecal excretion of 2-butoxyethanol: 2 – 3 % of administered dose - Major pathway of excretion: via urine (most of the radioactivity was excreted during the first 24 hours after dosing) - Higher urinary excretion (70 %) in 125 mg/kg bw group compared to 500 mg/kg bw treatment (40 %) - Two major metabolites detected: BAA and Glucuronide conjugate of	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Ghanayem et al. (1987c)

Method	Results	Remarks	Reference
or 500 mg/kg bw Collection of urine, faeces and expired air during 48 h after administration. Monitoring of biliary excretion of 2-butoxyethanol. Determination of radioactivity in each organ and in blood after 48 h.	2-butoxyethanol (BEG) - Two minor metabolites in urine: Sulfate conjugate of 2-butoxyethanol (BES) and one unknown substance - 2-butoxyethanol measured in urine at low concentrations - Between 8 and 24 h after dosing ~ 90 % of 2-butoxyethanol derived radioactivity was BAA (both treatments) - BAA, BEG and 2-butoxyethanol were excreted in bile - Organs with most radiolabeling: forestomach, liver and kidneys. 125 mg/kg bw; urine: - 2-butoxyethanol and BES only found within first 8 h after treatment - BAA only metabolite detected in urine (24 – 48 h) 500 mg/kg bw; urine: - 2-butoxyethanol and BES not detectable at any time - BAA/BEG ratio in urine: 3 to 1 - significant increases in bile flow as early as 0.5 h after administration (returned to normal after 4 h) - Biliary excretion of radioactivity continued to increase in a manner parallel with the increase in bile flow. - Cumulative excretion reached 8 % in 8 h - 2-butoxyethanol only detectable during first 2 h after treatment - BEG major metabolite excreted in bile.		
Metabolic study *In vivo* No TG followed No GLP compliance (study considered reliable with restrictions) **Fischer 344 rats** - Exposure by gavage First group: - Exposure duration: single doses of radiolabeled 2-butoxyethanol - Exposure dose/conc.: 500 mg/kg bw	Single exposure: - Exhalation of ~11 % of the 500 mg/kg dose as 14CO2 within 24 h after dosing - BAA was the major metabolite collected whatever the collection period (75 – 90 % of the total urine radioactivity) - Most of the remaining metabolite: glucuronide conjugate of 2-butoxyethanol - No sulfate conjugate detected - Lower biliary excretion of 2-butoxyethanol derived radioactivity than in pre-treated animals - Similar metabolic profile in bile compared to urine (BAA: 10, 21 and 46 % of total radioactivity excreted in bile fractions at 0 to 1, 2 to 4 and 6 to 8 h after treatment	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Ghanayem et al. (1987b)

Method	Results	Remarks	Reference
Second and third group: - Pre-treatment with 250 mg/kg bw pyrazole or cyanamide Intra Peritoneal (IP) - Second dose after 1 h - Exposure dose/conc.: 500 mg/kg bw radiolabeled 2-butoxyethanol Collection of urine (8, 24 and 48 h after dosing) and faeces (24 and 48 hr after dosing). Expired volatiles and $14CO_2$ were collected over 48 h. Determination of biliary excretion and radioactivity after treatment. Qualitative and quantitative determination of metabolites in urine and in bile.	Pre-treatment, pyrazole: - significant decline in % of 2-butoxyethanol dose exhaled as $14CO_2$ - significant increase in urinary excretion of 2-butoxyethanol derived radioactivity - Major metabolite: BEG (75 – 85 % of total radioactivity) - 8 – 19 % of the radioactivity was sulfate conjugate (not detected in rats treated with 2-butoxyethanol only) - Increase of biliary excretion more important than in 2-butoxyethanol only animals. - Higher biliary excretion of 2-butoxyethanol derived radioactivity (16 % vs 8 % for animals treated with 2-butoxyethanol only) - No BAA detected in bile - ~12 % of the radioactivity excreted in the 1 h as unchanged 2-butoxyethanol - Remaining portion: BEG - No metabolite other than BEG detected in bile fractions at 2 - 4 and 6 to 8 h after dosing with 2-butoxyethanol. Pre-treatment, cyanamide: - same results than with pyrazole. Conclusion: metabolism of 2-butoxyethanol to BAA is mediated by alcohol and aldehyde dehydrogenases via formation of BAL.		
Metabolic and disposition study *In vivo* No TG followed No GLP compliance (study considered reliable with restrictions) **Fischer 344 rats** - Young rats (4-5 weeks) and adults (9-13 weeks) - Oral administration - Exposure dose/conc.: 500 mg/kg bw - Exposure duration: single exposure	BAA, BEG and BES were identified in the urine of 2-butoxyethanol treated rats of either age. No BES was detected in the urine of either age group. An unknown metabolite was detected in the urine of both age groups. Young rats vs. adults: - Significantly higher % of 2-butoxyethanol exhaled as CO_2, 48 h after dosing in young rats (22 vs. 10 % of administered dose) - Significantly higher % of 2-butoxyethanol excreted via urine in young rats than adults - Significantly less BAA exctreted in young rats than adults - More BEG excreted via urine in young rats - Lower levels of 2-butoxyethanol derived radioactivity in all tissues of	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Ghanayem et al. (1987a)

Method	Results	Remarks	Reference
	young rats compared to tissues of adults.		
Metabolic and excretion study *In vivo* No TG followed No GLP compliance (study considered reliable with restrictions) **Fischer 344/N rats** - Males - Oral administration (drinking water) - Access to 2-butoxy [U-14C]ethanol (50 mCi/ mmol) for 24 h - Exposure doses/conc.: 290 ppm (237 µmol/kg bw), 860 ppm (401 µmol/kg bw) and 2590 ppm (1190 µmol/kg bw). - Exposure duration: single exposure In parallel 2 others rat groups were dosed with Ethylene Glycol Methyl Ether (EGME) and Ethylene Glycol Ethyl Ether (EGEE) for comparison with 2-butoxyethanol. Collection of exhaled CO2, urine and faeces during 72 hours from the beginning of the exposure. After collection period: determination of amount of radioactivity remaining in the cage, and total amount of water consumed by each rat. Rat carcasses analysed for total 14C. Urine was analysed for parent compound and metabolites.	80 % of excreted radioactivity during first 24h. Major metabolite identified: BAA (50-60 %, relatively constant for all doses). Other compounds identified: ethylene glycol, the glucuronide conjugate of 2-butoxyethanol and 2-butoxyethanol. No N-acetylglycine conjugate of BAA was identified in this study.	2-butoxyethanol (CAS-No.: 111-76-2) (purity: 99.9 %)	Medinsky et al. (1990)
Metabolic and disposition study (coupled with haematotoxicity study) *In vivo* No TG followed No GLP compliance (study considered reliable with restrictions) **Fischer 344 rats** - Males - Exposure by gavage	No quantitative or qualitative alteration of 2-butoxyethanol metabolism and disposition were caused by repeated exposure compared to single exposure. No difference in ratio of BAA, glucuronide and sulfate conjugates and parent 2-butoxyethanol excreted in urine of rats treated for 4 or 8 days compared to single exposure. Conclusion: tolerance development to the haemolytic effects of 2-butoxyethanol unlikely caused by increased	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Ghanayem et al. (1992)

Method	Results	Remarks	Reference
- Exposure regimen: 125 mg/kg bw for 3 or 7 days followed by a single dose of 125 mg/kg bw of 14C 2-butoxyethanol on day 4 and 8, respectively. Rats were placed in metabolism cages and 2-butoxyethanol metabolism analyses were performed.	detoxification of 2-butoxyethanol or inhibition of 2-butoxyethanol metabolism to BAA.		
Single dose exposure study *In vivo* No TG followed No GLP compliance (study considered reliable with restrictions) **Fischer 344 rats** - 3 rats/dose - Oral exposure by gavage - Vehicle: drinking water - Exposure dose/conc.: 500 mg/kg bw - Controls were given the same amount of water - Exposure duration: not specified The animals were killed 2 hr after exposure. The liver was excised and analysed for radiolabelled lipids.	~16 % of total radioactivity detected in liver associated with lipids (85 % of total lipids in phospholipid fraction). 3 % radioactivity of total lipids detected in ester fraction.	2-butoxyethanol (CAS-No.: 111-76-2) (purity: 99 %)	Kaphalia et al. (1996)
Metabolic study *In vivo* No TG followed No GLP compliance (study considered reliable with restrictions) **Sprague Dawley rats** - 3 sets of 4 treatments with 10 rats/group - Controls received water only. - Exposure by gavage - Exposure regimen: *First treatment*: alcohol only (10 or 30 mmol/kg) in distilled water, except for n-butanol (no vehicle), this group served as alcohol control group.	2-butoxyethanol only (5 mmol/kg): - Significant decrease of RBC (26 %) - Large increase in free plasma haemoglobin (Hb) concentration Co-administration of n-BuOH or n-PrOH (10 mmol/kg bw) and 2-butoxyethanol: - Partial reduction of haemolytic effect of 2-butoxyethanol - no changes in 24 hr urinary excretion of BAA Co-administration of EtOH (10 mmol/kg bw) and 2-butoxyethanol: - No haemolytic effect detectable - No changes in 24 h urinary excretion of BAA At the dose of 30 mmol/kg bw, each of the three alcohols co-administrated with 2-butoxyethanol produced a complete	2-butoxyethanol (CAS-No.: 111-76-2) (purity: 99 %)	Morel et al. (1996)

Method	Results	Remarks	Reference
Second treatment: 2-butoxyethanol only at 5 or 1 mmol/kg. *Third treatment*: simultaneous exposure to alcohol and 2-butoxyethanol (at the same doses than group 1 and 2 in distilled water (except for n-butanol: no vehicle). Haemolytic effect of 2-butoxyethanol (5 mmol/kg bw) was evaluated 4 hr after treatment by Red blood cell (RBC)-counting. Urinary concentration of BAA was determined on urine collected during 24 hr.	protection against haemolytic effects. BAA excretion changed, decreases of 43, 33 and 31 % were observed for EtOH, PrOH and BuOH, respectively. Alcohol control treatment did not have any effect on the haematological parameters. Urinary excretion of BAA in rats treated only with 1 mmol/kg bw 2-butoxyethanol was 0.083 mmol/24 h (~ 30 % of ingested dose).		
Accumulation/pharmacokinetics studies (5 experiments) No TG followed No GLP compliance (study considered reliable with restrictions) Endpoints examined: - **target tissue histology/ forestomach irritation** - **tissue dosimetry and pharmacokinetics** **B6C3F1 mice** - Females - 30/group - Exposure by IP injection (in saline solution) or gavage - Exposure doses/conc.: 50 or 250 mg/kg bw Blood collection after exposure and then the mice were killed 0.5, 1, 3, 6, 9, 12 and 24 h after dosing. Kidney, liver and stomach tissues were rapidly collected at each time point. The AUC and kinetic parameters for both 2-butoxyethanol and BAA were calculated.	2-butoxyethanol concentration in tissues paralleled the levels in blood regardless of dose or exposure route. For the 250 mg/kg dose to either route, concentration of 2-butoxyethanol higher and persisted longer in forestomach than in blood or in other tissues. Regardless of the route, $T_{1/2}$ and AUC higher in forestomach than in other tissues. $T_{1/2}$ for BAA: 2.1 h after gavage. By 24h, about 50 % of the total dose was eliminated in the urine (48 % for oral route) as 2-butoxyethanol, BAA or a conjugate. Following gavage administration of 2-butoxyethanol, BAA was major urinary metabolite (38 % of the dose). Small quantity (less than 0.2 %) of free 2-butoxyethanol, a conjugate of 2-butoxyethanol (up to 3 % - probably glucuronide) and a conjugate of BAA (between 0 and 7 %). Conclusion: 2-butoxyethanol can distribute to the forestomach by multiple mechanisms: grooming of the fur, mucociliary clearance, saliva and from systemic blood circulation. BAA can also distribute to forestomach tissues from saliva and blood circulation as well as being formed locally from 2-butoxyethanol.	2-butoxyethanol (CAS-No.: 111-76-2) (purity: 99 %)	Poet et al. (2003)
Metabolism and distribution study *In vivo*	- 50 fold more 2-butoxyethanol in forestomach than in blood or liver 5	2-butoxyethanol (CAS-No.: 111-76-2)	Deisinger and

Method	Results	Remarks	Reference
No TG followed No GLP compliance (study considered reliable with restrictions) **B6C3F1 mice** - Males and females - Oral administration (drinking water) - Exposure dose/conc.: 600 mg/kg - Exposure duration: single exposure - Control animals treated with distilled water At 5, 15, 45 and 90 min following dosing (5 min only for controls) animals were sacrificed. Collection of blood, liver and forestomach samples (analysed for 2-butoxyethanol, BAL and BAA).	min after dosing - Rapid elimination from blood and liver (but still measurable at 90 min post dosing) - 2-butoxyethanol concentration in forestomach decreased gradually (62 and 31 % of dose measured 5 min after dosing) - Low BAL concentrations in all organs (10fold higher in forestomach than blood or liver) - BAA increased until 90 min after dosing - Lower BAA concentrations in the forestomach than in blood or liver (at all time points) - No sex differences for 2-butoxyethanol and BAA concentrations in organs - Higher BAL organ concentrations in females than males (at all time points).	(purity: > 99 %)	Boatman (2004)
Toxicokinetic study No TG followed No GLP compliance (study considered reliable with restrictions) **B6C3F1 mice** - Males and females (5/sex/group) - Exposure by gavage (100 %; no vehicle) - Exposure doses/conc.: 100, 400, or 800 mg/kg-day (the 100 mg/kg-day treatment was increased to 1200 mg/kg-day after 2 days) - Exposure duration: daily for 1 week Study was terminated after 4 exposure days due to high mortality.	Severe haemolysis and mortality. Forestomach lesions: focal areas of irritation and epithelial hyperplasia at all exposure levels. Conclusions: In comparison with inhalation study, forestomach tissues show a similar irritative response whether 2-butoxyethanol exposure is systemic or portal of entry.	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Corley et al. (1999)
Inhalation			
Toxicokinetic inhalation study No TG followed No GLP compliance (study considered reliable with restrictions)	Absorption: - No differences in respiratory rate and tidal volume from unexposed rats - Amount of 2-butoxyethanol inhaled was proportioned to the exposure concentration in the 5 and 50 ppm groups, less than proportional amount was inhaled at 450 ppm due	2-butoxyethanol (CAS-No.: 111-76-2) (purity: 99 %)	Sabourin et al. (1992b)

Method	Results	Remarks	Reference
Fischer 344 rats - Males - Inhalative exposure (nose-only) - vapour - Exposure doses/conc.: 0, 0.024, 0.24, 2.18 mg/L (equivalent to 0, 5, 50, 450 ppm) - Exposure duration: 6h Determination of fractional uptake of inhaled 2-butoxyethanol and body burden of compound at 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 in a 2. 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. 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.	to a lower minute volume. Distribution: - Majority of 2-butoxyethanol equivalent was in plasma - First 2 h of exposure: 20 % of blood 14C associated with the red blood cell fraction - Later time points: proportion of 14C in the cellular fraction declined (undetectable after exposure) - Bound 14C metabolites greater after than during the exposure Identified metabolite: BAA (major), butoxyethanol glucuronide (BEG, minor), ethylene glycol (EG, minor), 2 further unidentifiable minor metabolites Excretion via urine: - Majority of 14C eliminated via urine - < 7 % of the parent compound was exhaled following the exposure - 10-20 % of (14C)2-butoxyethanol equivalent remained in carcass up to 66 h post exposure - 7 % of excreted 14C in the form of $14CO_2$ - More than 88 % of the total 14C excreted in urine during the first 41 h - BAA was the major metabolite in urine - EG and BEG were 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) favoured during the exposure - metabolism to BAA and ethylene Glycol (EG) favoured post exposure		
Toxicokinetic inhalation study *In vivo* No TG followed No GLP compliance	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.	2-butoxyethanol (CAS: 111-76-2) (purity: 99 %)	Johanson (1994)

Method	Results	Remarks	Reference
(study considered reliable with restrictions) **Sprague Dawley rats** - Males, 16/group - Continuous inhalative exposure (whole body) - vapour - Exposure 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)	Average tissues 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 tissues concentrations were approximately 5 times higher in blood, 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 *In vivo* No TG followed No GLP compliance (study considered reliable with restrictions) **Fischer 344 rats** - Males and females - Inhalative exposure (whole body, 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 after 2 weeks and 3, 6, 12	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	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %)	Dill et al. (1998)

Method	Results	Remarks	Reference
and 18 months of exposure.	of BAA in females, not depending on dose). Excretion rate of BAA tended to decrease with exposure time.		
Toxicokinetic inhalation study *In vivo* No TG followed No GLP compliance (study considered reliable with restrictions) **B6C3F1 mice** - females - Inhalative exposure (whole body, vapour) - Exposure dose/conc.: 1.2 mg/L (250 ppm) - Exposure duration: 6 h 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: 5 min after exposure: - 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 radiolabeling in the forestomach 24 h after exposure: - 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. 48 h after exposure: - high levels of radiolabeling 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 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	2-butoxyethanol (CAS: 111-76-2) (purity: 97.6 %)	Green et al. (2000)

Method	Results	Remarks	Reference
	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 *In vivo* No TG followed No GLP compliance (study considered reliable with restrictions) **B6C3F1 mice** - Males and females - Inhalative exposure (either whole body or nose-only, vapour) - Exposure does/conc.: 1.2 mg/L (250 ppm) - Exposure duration: 6 h 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. - High free BAA levels (about 2020 µg and 1780 µg for whole-body exposure and nose-only exposure, respectively)	2-butoxyethanol (CAS: 111-76-2) (purity unknown)	Poet et al. (2003)
Toxicokinetic inhalation study *In vivo* No TG followed No GLP compliance (study considered reliable with restrictions) **B6C3F1 mice** - Males and females - Inhalative exposure (whole body)	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-	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %)	Dill et al. (1998)

Method	Results	Remarks	Reference
- Exposure duration: 6h/day, 5 days/week, 104 weeks - Exposure doses/conc.: 0.3, 0.6, 1.2 mg/L (equivalent to 62.5, 125 and 250 ppm) 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.	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). Elimination of 2-butoxyethanol and BAA in aged mice: - 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 - 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		
Dermal			
Dermal absorption study *in vivo* and *in vitro* No TG followed No GLP compliance (study considered reliable with restrictions) *In vivo:* **Sprague Dawley rats** - Males - Occlusive application using a glued circular ring covered with membrane cap - Exposure duration: 4 h - Exposure doses/conc.: 50 µL/cm^2 of neat or 20 – 95 % aqueous solution of	Percutaneous absorption rate: - Flux of 50% solution (maximum seen): 1.38mg/cm2/h ± 0.16 % - Flux of neat solution: 0.40mg/cm2/h ± 0.06 %. Thermodynamic activity for neat 2-butoxyethanol and water in aqueous solutions of 2-butoxyethanol: - skin fully hydrated and flux of 2-butoxyethanol through it is proportional to the thermodynamic activity of 2-butoxyethanol - Exception: when the water content in the vehicle is small - reduced 2-butoxyethanol flux arising from skin dehydration - Previously published data: activity-normalised flux of 2-butoxyethanol through hydrated human skin is 2 – 4 mg cm^{-2} h^{-1}	2-butoxyethanol (CAS: 111-76-2) (purity unknown)	Bunge et al. (2012)

Method	Results	Remarks	Reference
[14C]2-butoxyethanol; Collection of urine, faeces, and air ([14C]2-butoxyethanol and CO_2 using a water trap) After exposure, animals were euthanized. Absorption flux was measured in 4 – 6 rats at each exposure concentration. *In vitro:* Absorption through silicone membranes and rat skin in static diffusion cells with a diffusion area of 1.76 cm^2. Neat or aqueous solutions (1–90%; V/V) of [14C]2-butoxyethanol (200µL/cm^2) applied onto skin or silicone membranes, occluded with paraffin film. Measurements of radioactivity and thermodynamic activity.			
Dermal absorption study *in vivo* (and *in vitro*) No TG followed No GLP compliance (study considered reliable with restrictions) **Wistar rats** - Males - Occlusive application - Exposure duration: 24 h - Exposure dose/conc.: nominal dose 100µl (10.53 µCi) The dermis from topical application sites of the rats killed at 4 and 24h was analysed to determine levels of radioactivity distribution, metabolism and elimination. Unexposed skin was used as a control. *In vitro* methods and results not reported here.	Absorption (after 24 h): - Non-occlusive cover (including charcoal filter and air + enclosure rinse): 56.2% - Skin wash: 0.3% - Skin test site: 0.1% - Blood: 0% - Carcass+liver: 1.6% - Urine: 18.8% - Cage wash + cage wipe: 3% - Faeces: 1.5% - Expired air (if applicable): 5.7% Fate of absorbed dose (after 24h) - Skin test site: 4.3% - Blood: 0% - Carcass: 1.6% - Liver: 4.7% - Urine: 66% - Cage wash + cage wipe: 0.9% - Faeces: 0.4% - Expired air (if applicable): 20% Total recovery: 85-90% over all time points	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Lockley et al. (2004)
Toxicokinetic study, cutaneous absorption *In vivo* No TG followed No GLP compliance (study considered reliable with	Experiment 1: - Within 48 h following topical application: 20 to 23 % of applied radioactivity found in urine - 95 % eliminated during the first 24 h - Percutaneous absorption of 25 – 29 % of applied topical dose	2-butoxyethanol (CAS: 111-76-2) (purity: 99 %)	(Bartnik et al., 1987)

Method	Results	Remarks	Reference
restrictions) Experiment 1: **Wistar rats** - 6 males and 6 females - Topical application on 12 cm^2 of shaved skin, site kept covered with perforated glass capsule - Exposure duration: 48 h - Exposure doses/conc.: 200 mg/kg bw Urine collection for 48 hours. After termination, treated skin area was dissected for determination of radioactivity. Assessment of percutaneous absorption via measurements of 14C in urine from 0 to 48 h following administration. Experiment 2: **Wistar rats** - 24 females - Topical application on 12 cm^2 of shaved skin - Exposure doses/conc.: 200 mg/kg bw Animals were killed 0.5, 1, 2, 4, 6, 8, 16 and 24 hours after application. Measurements of 2-butoxyethanol and BAA in blood.	Experiment 2: - highest radioactivity in blood and plasma at 2 h following application - Based on BAA levels in the plasma, it is suggested that the majority of absorbed 2-butoxyethanol is metabolised to BAA.		
Dermal absorption study *in vivo* No TG followed No GLP compliance (study considered reliable with restrictions) **Fischer 344/N rats** - Males - non occlusive application - Exposure duration: 72 h - Exposure doses/conc.: 122, 367 and 650 µmol/rat (equals 60, 182, 322 mg/kg bw) Collection of 2-butoxyethanol, CO_2, urine and faeces for 72 h. After 72 h, rats were killed and skin around dosing site was removed. Digested tissues, urine and faeces were assayed for radioactivity.	Absorption: - 43 to 64 % of the dermally applied dose trapped as volatile 14C - 20 - 25 % of the dermally applied dose absorbed and metabolised - 0.3 - 2 % of the dermally applied dose still present at application site 72 h following dosing - 7 - 16 % of absorbed radiolabel remained in the carcass at the end of the collection period Excretion: - Majority of radioactivity excreted in urine - Small amounts of 14C found in faeces - Small amounts of 14C exhaled as CO_2 - exposure concentration did not affect excretion of 14C, except for a slight increase in the proportion of $14CO_2$	2-butoxyethanol (CAS: 111-76-2) (purity: 99 %)	Sabourin et al. (1992a)

Method	Results	Remarks	Reference
	Urinary metabolites: - BAA major urinary metabolite - Detection of glucuronide conjugates - Over 80 % of radioactivity associated with plasma - Maximum concentration of total plasma metabolites 1 h following dermal application of 367 µmol - concentration of total plasma metabolites decreased with a half-life of about 4 h at 367 µmol - 53 - 75 % of plasma 14C attributable to plasma BAA		
Dermal absorption study *in vivo* No TG followed No GLP compliance (study considered reliable with restrictions) **Guinea Pigs** - Females (kept under anesthesia throughout the experiment) - *Intra Venous* (IV) bolus, followed by percutaneous application - Exposure doses/conc.: IV: 42 or 92 µmol/kg bw; percutaneously: undiluted test substance Blood was sampled after 5, 10, 20, 30, 40, 50, 60, 75, 90, 105 and 120 min after IV. At 150 min after IV dosing, animals were occlusively exposed to 1 ml of undiluted 2-butoxyethanol percutaneously (3.14 cm2 of skin exposed). Blood samples were collected after 5, 10, 20, 30, 40, 50, 60, 75, 90, 105 and 120 min.	IV administration: - Rapid decline of 2-butoxyethanol in blood after IV. - Total clearance estimates (Cl): 128 ml/ min/kg bw corresponding to 2.7 ml/min/g of liver (0.8 ml/min/g of liver in human and 2 ml/min/g of liver in perfused rat liver). Dermal administration: - Rapid rise of 2-butoxyethanol concentration in blood. - Plateau level during second half of exposure period (average 21 µmol/l) - Estimated average uptake rate: 0.25 µmol/min/cm^2.	2-butoxyethanol (CAS: 111-76-2) (purity unknown)	Johanson and Fernstrom (1986)
Dermal absorption study (influence of water on percutaneous absorption) *in vivo* No TG followed No GLP compliance (study considered reliable with restrictions) **Guinea Pigs**	Concentration of 2-butoxyethanol in blood increased with time after administration. The relative percutaneous uptake rates were approximately equal from the 5, 10, 20, and 100% solutions of BE, while they were approximately twice as high from the 40 and 80% solutions. 2-butoxyethanol concentration in blood declined ~ twice as rapidly after the end of exposure to the 40 and 80 % solution (average $t_{1/2}$: 28 min vs. 55 min for the	2-butoxyethanol (CAS: 111-76-2) (purity unknown)	Johanson and Fernstrom (1988)

Method	Results	Remarks	Reference
- Animals kept under anesthesia for 6 h - Duration of first semi-occlusive epicutaneous exposure: 2 h - Exposure doses/conc.: 100, 80, 40, 20, 10, and 5 % - 2 h recovery period - Duration of second epicutaneous exposure: 2 h - Exposure dose/conc.: 100 % - Skin uptake of diluted test substance was compared with skin uptake of undiluted test substance, each animal serving as its own control. Arterial blood collected at intervals of 10 to 30 min throughout the experiment.	other solutions). Recovery period: After removal of exposure source, concentration decreased immediately. Second exposure: During the 2nd hour of exposure to pure 2-butoxyethanol, blood level appeared to approach a plateau level. Average concentration during the last hour: 4.6 µmol/l. Calculated skin uptake rate: 132 nmol/min/cm^2.		

Method	Results	Remarks	Reference
Cutaneous absorption study, modified method of Zesch and Schaefer (1973) *In vitro* No TG followed No GLP compliance (study considered reliable with restrictions) - Evaluation of cutaneous absorption under semi occlusive and non-occlusive conditions. - **Skin of rats, pigs and humans** - Before and at end of each experiment, skin was checked visually for integrity of stratum corneum - For application of test material (30 µL) on 5 cm^2 of animal skin or 3 cm^2 of human skin - Exposure doses/conc.: 100, 10, and 3.5 % (aqueous solution) Test solutions were evaluated for absorption in rat skin (1, 6 and 16 h semi-occlusive, 1h exposure non-occlusive) and pig skin (6 h exposure semi-occlusive). Absorption of 3.5 % 2-butoxyethanol were assessed on both rat and pig skin under non-occlusive conditions (10, 30 and 60 min exposure). Samples of rat, pig and human skin were treated with 10 % 2-butoxyethanol (1h exposure, semi-occlusive and non-occlusive conditions).	Semi-occlusion in vitro: - 2-butoxyethanol readily absorbed and completely absorbed after 16 h of exposure. - Penetration depends on time as well as on concentration. - Penetration rate of pure 2-butoxyethanol slower than from aqueous solutions, but more complete after 16 hr. - After 6 h under semi-occlusive conditions: penetration through pig skin was 2 or 3 times less rapid than through rat skin. - Application of 2-butoxyethanol on rat skin under non-occlusive conditions: great reduction in absorption due to volatility of the compound. - Within 10 min following application, a major proportion of the absorbed material had penetrated. - 2 fold higher absorption in rat skin compared to pig skin. - Human skin: under semi-occlusive condition, penetration rate comparable with that through pig skin and much slower than through rat skin. - Under non-occlusive conditions, human skin exhibits the lowest percutaneous absorption (6.9 % of the applied dose).	2-butoxyethanol (CAS: 111-76-2) (purity unknown)	Bartnik et al. (1987)
Percutaneous absorption study *In vitro* (and *in vivo*) No TG followed No GLP compliance (study considered reliable with restrictions) - **Skin of rats and humans** - Exposure to undiluted 2	*In vitro:* Toxicokinetic parameters: - Rat whole skin Km: 0.56+/-0.06 mM, - Rat liver Km: 1.5+/-0.6 mM, - Rat whole skin Vmax: 15.5+/-1.7 nM NADH/min/mg protein - Rat liver Vmax: 3.3+/-2.9 nM NADH/min/mg protein. Details on metabolites: - Evidence of conversion of NAD to	2-butoxyethanol (CAS: 111-76-2) (purity unknown)	Lockley et al. (1999) and Lockley et al. (2004) and Lockley et al. (2005)

Method	Results	Remarks	Reference
butoxy [1-14C] ethanol (1.41 mg/cm2) - Occlusive application Skin surface swab (unabsorbed material), tape strip (material in the stratum corneum), skin (material in the epidermis and remaining dermis) and absorbed dose (receptor fluids in vitro and mass balance cumulative dose *in vivo*) were analysed up to 24 h after application. The metabolic capacity of the skin was also examined *in vitro*. Evaporated 2-butoxyethanol was trapped with carbon filters (> 80 % of the dose within 1 hr of application). *In vivo* methods and results not reported here. The results of the *in vitro* and *in vivo* study were compared.	NADH suggesting oxidation of 2-butoxyethanol to BAA. - No bioaccumulation potential based on study results. Absorption: - Rapid absorption of 2-butoxyethanol; - lag phase of approx. 1 h following which a steady state resulted - Significant evaporation (70-80% recovered in the carbon filters) - Dissolution in methanol solvent enhanced rate of permeation, but not markedly. Absorption of 2-butoxyethanol *in vitro* through rat skin most closely reflected penetration *in vivo*. Absorption through human skin *in vitro* was less than rat skin but reflected dermal absorption described in a previous study (Johanson et al., 1988). Conclusion: *in vitro* studies for 2-butoxyethanol reflect *in vivo* conditions.		
Other routes			
Degradation/elimination study *In vivo* No TG followed No GLP compliance (study considered reliable with restrictions) **Sprague Dawley rats** - Females - 4/group - Administration intraperitoneal (IP) - Exposure doses/conc.: 2.5 mmol/kg bw or 2.5 mmol/kg bw simultaneously with 20 mmol/kg EtOH.	Degradation of 2-butoxyethanol almost completely inhibited when simultaneously treated with EtOH (elimination: - without EtOH: 40 min - with EtOH: 150 min). EtOH elimination rate 5 – 6 % slower in presence of 2-butoxyethanol.	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Römer et al. (1985)
Metabolism and elimination study *In vivo* No TG followed No GLP compliance (study considered reliable with restrictions) **Wistar rats** - Males	- 78 % of radioactivity in urine within 72 h - < 1 % in faeces - 10 % exhaled as CO_2 - 1.6 % absorbed on activated charcoal indicating that the exhaled air contained only a small amount of the test compound or volatile metabolites - At the end of the experiment, 4.8 % of the radioactivity	2-butoxyethanol (CAS-No.: 111-76-2) (purity: 99 %)	Bartnik et al. (1987)

Method	Results	Remarks	Reference
- 3/group - Subcutaneous administration of radiolabeled 2-butoxyethanol - Exposure dose/conc.: 118 mg/kg Collection of faeces, urine and expired air during a 72-hour period following dosing. Collection of liver, kidneys, spleen, fat, testes, thymus, sternum (including bone marrow) and Blood after termination.	found in carcass - Highest radioactivity in spleen and thymus followed by liver.		
Toxicokinetic studies (series of experiments) *In vivo* No TG followed No GLP compliance (study considered reliable with restrictions) **Fisher 344 rats** - Males, young (3-4 weeks) and adult (12-13 months) - 3-4/group - Intravenous administration - Exposure regimen: First group: - Exposure duration: single doses of radiolabeled 2-butoxyethanol - Exposure dose/conc.: 500 mg/kg bw Second and third group: - Pre-treatment with 250 mg/2.5 mL 0.9 % saline/kg pyrasole or 50 mg/mL 0.9 % saline/kg cyanamide via IP - Exposure after 20 – 30 min - Exposure dose/conc.: single bolus of 31.25, 62.5 or 125 mg/kg bw radiolabeled 2-butoxyethanol (IV) - Fourth group: - Pre-treatment probenecid (50 mg/2.5 mL of 0.9 % saline alkalinised with NaHCO3/kg) via IP - Second dose of probenecid	General observations: - Higher concentration of radioactivity in plasma than whole blood - Only 2-butoxyethanol and BAA in the plasma, no BEG - No significant effect of dose on $T_{1/2}$ or Vd (Volume of distribution) but on C_{max}, AUC (both decreased) and Cl (increased) of 2-butoxyethanol - Significant increase in $T_{1/2}$ of BAA in adults (low and high dose, not middle dose). Effect of dose and age: - 2-butoxyethanol in blood is proportional to the administrated dose. - Higher 2-butoxyethanol concentrations in adult vs. young animals - No effects of age on $T_{1/2}$ - Increased C_{max} and AUC of 2-butoxyethanol in adult rats - Increased C_{max} and AUC of BAA in adult rats Effect of alcohol dehydrogenase inhibition by pyrazole pre-treatment: - Total radioactivity lower at all time points and at both dose - BE-glucuronide conjugate (BEG) detected in plasma - Significant increase in $T_{1/2}$, AUC and Cl of 2-butoxyethanol at both dose levels - No effect on Vd - Significant decrease of AUC, Cmax and t1/2 for BAA Effect of aldehyde dehydrogenase inhibition by cyanamide pre-treatment: - Higher radioactivity in whole blood than plasma at all time points beyond	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Ghanayem et al. (1990)

Method	Results	Remarks	Reference
after 4 h - Exposure after 20 – 30 min - Exposure dose/conc.: single bolus of 31.25, 62.5 or 125 mg/kg bw radiolabeled 2-butoxyethanol (IV) Collection of blood samples at 5, 15 and 30 min and at 1, 2, 4, 6 and 8 h. Determination of 2-butoxyethanol and metabolites in whole blood and plasma.	the first 30 min after 2-butoxyethanol administration - Lower total plasma radioactivity at all time points - Increase of $T_{1/2}$, AUC, Vd and Cl of 2-butoxyethanol - No effect on Cmax - Slightly higher $T_{1/2}$ of BAA - Significant decrease of the C_{max} and AUC of BAA Effect of inhibition of renal tubular anion transport by probenecid pre-treatment: - Higher total radioactivity in plasma and whole blood - No changes in C_{max}, AUC, $T_{1/2}$, Vd or Cl of 2-butoxyethanol - Significant increase in $T_{1/2}$ of BAA (greatest at lower dose) - Significantly increase of AUC of BAA (greatest at lower dose) - No effect on C_{max} of BAA Conclusions: BAA is the proximate haemolytic agent and is formed from 2-butoxyethanol by a metabolic pathway involving alcohol and aldehyde dehydrogenase. The renal organic acid transport may play a role in the clearance of BAA. Higher sensitivity of older rats compared to younger rats concerning haematotoxicity due to a combination of factors: compromised renal clearance of BAA by the renal anion transport system in older rats, increased 2-butoxyethanol metabolism to BAA, diminished degradation of BAA to CO_2 and greater sensitivity of erythrocytes in older rats.		
Toxicokinetic studies *In vivo* No TG followed No GLP compliance (study considered reliable with restrictions) **B6C3F1 mice** - Females - Intravenous administration - Exposure dose/conc.: 10 mg/kg	After 4 h: - Highest concentrations of radiolabel in the liver, Harderian glands, salivary glands, nasal passages, oesophagus, buccal cavity and on the surface of the feet - Lower concentrations in the stomach, gastro intestinal (GI) tract contents and mucosa, kidney cortex and associated with bones - Background levels of labelling in lungs and remaining internal organs. After 24 h: - Highest concentrations of radiolabel in liver, bone, Harderian glands,	2-butoxyethanol (CAS-No.: 111-76-2) (purity: 97.6 %)	Green (2000)

Method	Results	Remarks	Reference
Animals were terminated at 4, 24 and 48 h after dosing (4/time point, one animal for whole body autoradiography, three animals for analysis of stomach and contents). Determination of radioactivity in forestomach, glandular stomach and stomach contents (free and bound radioactivity).	surfaces of the feet and buccal cavity - Slightly lower amounts in mucosa of the stomach, GI tract and oesophagus - Lower levels of labelling in salivary glands and kidney cortex. - Slight levels of labelling in spleen, GI content and the kidney medulla - Background labelling in lungs and rest of internal organs. After 48 h: - Highest concentrations of radiolabel in liver, bone, buccal cavity and oesophagus - Slightly lower levels in mucosa of the forestomach and glandular stomach - Lower levels in salivary glands and GI tract mucosa - Background labelling in lungs and remaining internal organs Total radioactivity in forestomach and glandular stomachs similar over the duration of the study. Most radioactivity being present in stomach walls: 80-95 % of the radioactivity bound to protein at the two later time points. Conclusions: Radioactivity in the stomach tissue appears to be derived from systemic circulation and ingestion from the buccal cavity (origin of the latter unknown, potentially derived from salivary and Harderian glands).		
Toxicokinetic studies regarding accumulation of the test substance in forestomach (5 experiments) *In vivo* No TG followed No GLP compliance (study considered reliable with restrictions) Endpoints examined: - **target tissue histology/ forestomach irritation** - **tissue dosimetry and pharmacokinetics** **B6C3F1 mice** - Females - 30/group	2-butoxyethanol concentration in tissues paralleled the levels in blood regardless of dose or exposure route. For the 250 mg/kg dose to either route, concentration of 2-butoxyethanol higher and persisted longer in forestomach than in blood or in other tissues. Regardless of the route, $T_{1/2}$ and AUC higher in forestomach than in other tissues. Max. BAA concentrations in blood, kidneys and liver during the first 3 h after IP administration. Thereafter, higher BAA concentrations in forestomach than in other tissues. BAA concentrations in glandular	2-butoxyethanol (CAS-No.: 111-76-2) (purity: 99 %)	Poet et al. (2003)

Method	Results	Remarks	Reference
- Exposure by IP injection (in saline solution) or gavage - Exposure doses/conc.: 50 or 250 mg/kg bw Blood collection after exposure and then the mice were killed 0.5, 1, 3, 6, 9, 12 and 24 h after dosing. Kidney, liver and stomach tissues were rapidly collected at each time point. The AUC and kinetic parameters for both 2-butoxyethanol and BAA were calculated.	stomach tissue similar to other tissues. Higher $T_{1/2}$ and AUC of 2-butoxyethanol after gavage than IP. $T_{1/2}$ for BAA: 1h for IP. By 24h, ~ 50 % of the total dose eliminated via urine (54 % for IP) as 2-butoxyethanol (less than 0.2 %), BAA found (50 % for the IP) or a conjugate (up to 3 %) for the 250 mg/kg doses. Last substance detected: conjugate of BAA (between 0 and 7 %).		
Toxicokinetic studies regarding accumulation of the test substance in forestomach (5 experiments) *In vivo* No TG followed No GLP compliance (study considered reliable with restrictions) Endpoint examined: - **salivary excretion** **B6C3F1 mice** - Females - 30/group - Exposure by IP injection (in saline solution) or gavage - Exposure dose/conc.: 250 mg/kg bw - Induction of salivation by injection of pilocarpine a few minutes before saliva collection Collection of saliva under anaesthesia at various times after administration (up to 2.5 hr; periods of 15 to 30 min). Collection of blood at the midpoint of each saliva collection interval and at the end of saliva collection. Determination of kinetic parameters of 2-butoxyethanol and BAA in blood and saliva.	Peak blood and saliva concentration of 2-butoxyethanol at 15 and 7.5 min, respectively, regardless of the route. Similar concentrations in blood and saliva at all time points (after 1.3 h below detection level). Similar AUC and $T_{1/2}$ for 2-butoxyethanol in blood and saliva. Time curve for BAA similar for blood and saliva, with saliva concentrations being 4 fold lower than blood levels. AUCs and $T_{1/2}$s in blood and saliva were higher for BAA than BE. 2x higher $T_{1/2}$ for BAA in blood than in saliva (in blood 1.4 and 1.6 h for IP and gavage, respectively).	2-butoxyethanol (CAS-No.: 111-76-2) (purity: 99 %)	Poet et al. (2003)
Toxicokinetic studies regarding accumulation of the test substance in forestomach (5 experiments) *In vivo*	Higher 2-butoxyethanol concentration in the stomach content than in forestomach tissue. No 2-butoxyethanol detected in either	2-butoxyethanol (CAS-No.: 111-76-2) (purity: 99 %)	Poet et al. (2003)

Method	Results	Remarks	Reference
No TG followed No GLP compliance (study considered reliable with restrictions) Endpoint examined: - **retention in stomach content** **B6C3F1 mice** - Females - 30/group - Exposure by IP injection (in saline solution) - Exposure dose/conc.: 250 mg/kg bw After sacrifice (3, 6 and 9 hours after exposure) 2-butoxyethanol and BAA were quantified in stomach tissue and stomach contents.	blood or glandular stomach tissue at any time point. The estimation of $T_{1/2}$ for 2-butoxyethanol in stomach content: 4.8 h after IP injections (longest of all tissues).		
Toxicokinetic study B6C3F1 mice - Males and females (3/sex/group) - Exposure by either intraperitoneal (IP) or subcutaneous (SC) injection - Exposure regimen: 400 or 600 mg/kg/day for 3 consecutive days or 0 and 400 mg/kg/day for 5 consecutive days	Focal irritation in the forestomach at 600 mg/kg for 3 days, while 1/3 mice at 400 mg/kg IP and at 400 and 600 mg/kg SC for 3 days also had forestomach lesions, minimal effects. At 400 mg/kg (5-day study), 1/6 mice (IP) and 2/6 mice (SC) also had minimal lesions. Conclusion: In comparison to oral exposure, forestomach tissues show similar irritative response if 2-butoxyethanol exposure is IP or SC.	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Corley et al. (1999)
Humans - oral			
Case report (suicide attempt) - 50year old woman - Ingestion of 250-500 mL window cleaner containing 12 % of 2-butoxyethanol (~ 0.5 - 1 g/kg bw).	- Comatose patient - Metabolic acidosis - Hypokalaemia - High serum creatinine level - Increased urinary excretion of oxalate crystals	2-butoxyethanol (CAS-No.: 111-76-2) (12 % in a formulation)	Rambourg-Schepens et al. (1988)
Case report (suicide attempt) - 18year old man - Ingestion of window cleaner ~ 360 - 480 mL (79 - 106 g or 1.1 - 1.5 g/kg)	- Max. BAA blood concentration 4.86 mmol/L - Metabolic acidosis - Hepatic biochemical disorders (Serum Glutamic Oxaloacetic Transaminase, Serum Glutamic Pyruvic Transaminase and hepatic bilirubin) - Signs of haemolytic anaemia	2-butoxyethanol (CAS-No.: 111-76-2) (in a formulation; % unknown)	Gualtieri et al. (2003)
Case report (suicide attempt) - 47year old man	- Mixed metabolic acidosis-respiratory alkalosis with marked anion gap (30mmol/L)	2-butoxyethanol (CAS-No.: 111-76-2) (in a formulation; %	Butera et al. (1996)

Method	Results	Remarks	Reference
- Ingestion of 500 mL of cleaning product (~ 0.5 g/kg; 340 mmol total dose)	- 2-butoxyethanol and BAA both blood plasma and urine - Metabolites in urine accounted for ~95% of the total dose (over 96hrs monitoring) - ~90% of ingested dose was accounted for by excreted BAA in urine - No haemolysis - Microhaematuria - Reduction in haemocrit and haemoglobin	unknown)	
Case report (accidental ingestion) - 16 months old girl - Ingestion of unknown amount of cleaning solution containing 10 – 30 % of - Further ingredients: monoethanolamine (5–10%), alkoxylated linear alcohols (1–5%), ethylenediaminetetraacetic acid (1–5%), potassium hydroxide (1–5%)	- Metabolic acidosis was manifest - No evidence of alkaline mucosal injury, hepatic or renal dysfunction, or haemolysis	2-butoxyethanol (CAS-No.: 111-76-2) (10 – 30 % in a formulation)	Osterhoudt (2002)
Case report (accidental ingestion) - 24 children - 7 months to 9 years - Ingestion of at least 5 mL of window cleaner containing 0.5 – 9.9 % 2-butoxyethanol - 2 children drank more than 15 mL	No symptoms of 2-butoxyethanol poisoning, such as metabolic acidosis, and no haemolysis.	2-butoxyethanol (CAS-No.: 111-76-2) (0.5 – 9.9 % in a formulation)	Dean and Krenzelok (1992)
Humans - 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	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).	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Johanson et al. (1986a)

Method	Results	Remarks	Reference
during light physical exercise (50W) Collection of blood samples during the exposure period and 3 h afterwards for determination of 2-butoxyethanol concentrations. Collection of urine for a period of 24 h. 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 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 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. Conclusions: 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	Urine analysis: Peak excretion 6-12 h post exposure. Mean half-life: 4h. Conjugation variable between individuals but does not slow elimination. Haematology: Mean peak blood concentration of 2-	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Jones and Cocker (2003)

Method	Results	Remarks	Reference
- 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. Collection of breath samples: at 0, and 2h, then every 10-15 min for further 2 h.	butoxyethanol: 7µM. Mean half-life: 56min. BAA: peaked 20 min after exposure (at average of 35µM). Mean half-life: 13 mins. Breath measurements: Maximum value only 12x LOD, so not deemed a reliable technique to quantify exposure.		
Inhalation studies, toxicokinetics Experiment 1: - 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 Experiment 2: - 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.	Experiment 1: One male and the 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. Experiment 2: 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. Haematology: No adverse effects seen at either exposure concentration.	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	The 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/m^3 or 0.27 ppm)	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	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown, incidental, occupational exposure)	Haufroid et al. (1997)

Method	Results	Remarks	Reference
- Co-exposure to methyl ethyl ketone - Use of an unexposed control group	normal clinical ranges.		
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)
Humans - dermal			
Skin penetration test with human epidermis *In vitro* Comparison of 2-butoxyethanol with 3 other glycol ethers. - Disks of human abdominal skin placed in diffusion chambers (n = 8) - Assessment of membrane integrity before the test as baseline by measurement of permeability to tritiated water. Measurement of glycol ether absorption rate for a period of 8 h. Determination of potential of tested substance to impair epidermal diffusion barrier function. Calculation of the damage ratio: Permeability constant after glycol ether contact / permeability constant before glycol ether contact.	Mean rate of penetration for undiluted 2-butoxyethanol: 0.20 mg/cm^2/hr (±0.03 SEM, n=8). 2-butoxyethanol did not produce large alterations in permeability. Damage ratio = 2.07. Conclusions: The measured ratio indicates a marginal effect with little damage to the skin following prolonged exposure to 2-butoxyethanol.	2-butoxyethanol (CAS-No.: 111-76-2) (purity: > 99 %)	ICI (1982a) and ICI (1982b)
Skin penetration test with human skin *In vitro* - Human abdominal skin (stratum corneum), n = 8 - Exposure via Franz-type diffusion cells - Exposure conc.: 100% - Exposure duration: not specified - 2 test trials Determination of integrity of the skin sample before and after exposure by measuring the	Mean absorption rates for 2-butoxyethanol: 0.857 mg/cm2/h ± 0.282 in the first experiment and 1.52 mg/cm2/h ± 0.37 in the second experiment (high variability). Mean damage ratio: 3.25 ± 3.33 in the first experiment and 5.14 ± 4.99 in the second experiment (high variability). Due to the high variability, mean absorption rates were calculated separately for the undamaged skin (n=8) and the damaged ones (n=4). Mean absorption rates for damaged skin	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Eastman Kodak (1991)

Method	Results	Remarks	Reference
permeability to tritiated water.	3 times higher than for undamaged skin (3.39 mg/cm^2/h vs. 1.19 mg/cm^2/h). When results from four cells showing high damage ratio are excluded: mean damage ratio: 1.66 ± 1.31.		
Percutaneous absorption test with human skin (following the recommendations of FDA, AAPS, COLIPA, SCCNFP and OECD for this kind of test) *In vitro* GLP compliant (study considered reliable with restrictions) - Human skin (1.76 cm^2) - Exposure conc.: 10 and 5 % in an oxidative hair dye formulation; 33 mg per formulation and test (corresponding to 20 mg/cm^2) - Exposure duration: 30 min - Washing of skin Monitoring of diffusion of 2-butoxyethanol 24 h following application. Collection of receptor fluid at 2, 4, 6, 10, 21 and 24 h after the beginning of exposure. After 24 h observation period, tissues (horny layer, epidermis and dermis) analysed for remaining 2-butoxyethanol.	Results showed that no accumulation of 2-butoxyethanol in skin occurred. For the two concentrations tested , the percentage of absorption were similar (12.1 and 12.5 % for the 5 % and 10 % concentrations, respectively).	2-butoxyethanol (CAS-No.: 111-76-2) (in a formulation; 5 and 10 %)	PMIC (2001)
Percutaneous absorption test with human skin *In vitro* - Human breast skin, full thickness or dermatomed (stratum corneum + upper dermis) - Exposure conc.: 3, 6 mg/L aqueous solution; 100 and 200 µL in skin, or undiluted (10.5 µL) Determination of percutaneous absorption 24 h using flow through diffusion cells. Tissue culture medium was used as a receptor fluid with 2 % (w/v) bovine serum albumin (BSA) or 2-6 % (w/v) polyethylene glycole 20 (PEG 20) added for some studies.	Total recovery: 88.5% (receptor fluid 27.4%, charcoal filter 60.6%, surface and cell washes 0.20%, skin 0.26%). Aqueous solutions: Steady state flux: 544 ± 64 nmol/cm^2/h (0.064 mg/cm^2/h) for dermatomed skin. Reduced dose (100 µL) decreased steady state flux by about 55 %; increased dose: raise to 894 ± 217 nmol/cm^2. Full thickness skin increased time to steady state (tau) and reduced steady state flux. Penetration rate at all concentrations similar at around 0.02cm/h. Undiluted : Absorption rates exceeded those measured for aqueous solutions, though	2-butoxyethanol (CAS-No.: 111-76-2) (purity: 98 %)	Wilkinson and Williams (2002)

Method	Results	Remarks	Reference
	the apparent permeability coefficient was higher with the aqueous doses. Maximum flux rate 8500nmol/cm^2/h (= 1.0 mg/cm^2/h).		
Dermal absorption study with human skin *In vitro* No TG followed GLP compliance not specified (study considered reliable with restrictions) - Human breast skin - Test method: diffusion cells glued to skin - Underside of skin in contact with receptor fluid (complete solubility of test substance in receptor fluid) - Dosing of skin surface with neat 2-butoxyethanol (115.2 mg) or 14C-butoxyethanol (115.2 mg, equivalent to 56 kBq/cell) Collection of receptor fluid from beneath the skin at 0, 4, 8, 12, and 24 h. Monitoring of absorption and metabolism of 2-butoxyethanol to BAA over time.	Total recovery: 56 % of the applied dose (unchanged or in the form of its metabolites) were removed from skin surface at 24 h. The equivalent of 17.5 % of the applied dose was recovered from receiver fluid, 3% from within the skin and the remaining 23.5 % of the dose was lost to the atmosphere through evaporation. After 24, only 0.03% of the applied dose had been metabolised to BAA. Thus, about 0.16 % of absorbed 2-butoxyethanol was metabolised to BAA during its passage through the skin. Permeation of 2 -butoxyethanol was linear with time with no discernible lag time (high interindividual variability). Presence of the retinol reduced the rate of production of BAA by about a third. Conclusions: Although enzyme activities capable of converting butoxyethanol to BAA are present in skin, metabolic conversion during percutaneous absorption is small and systemic exposure would occur rather to the parent compound rather than the metabolite following dermal exposure.	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Traynor et al. (2008) and Williams (2008)
Dermal absorption study with human skin *In vitro* According to OECD test guideline (TG) 428 (Skin Absorption: *In Vitro* Method) GLP compliance not specified (study considered reliable with restrictions) - Human skin from abdomen - Test method: diffusion cells glued to skin - Receptor fluid (0.9% NaCl solution in water; full solubility of test substance in receptor fluid) - Exposure conc.: 100% or 50% (v/v) aqueous solution under infinite	Percutaneous absorption rates: Neat compound: 45 ± 3µg/cm^2/h. 50% solution: 704 ± 33µg/cm^2/hr.	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Korinth et al. (2012)

Method	Results	Remarks	Reference
dose Collection of samples 8 h after exposure. Determination of percutaneous penetration rates as maximum fluxes using the slope of the linear regression of the cumulative mass of the compound penetrating into the receptor fluid per cm^2 of skin versus time.			
Percutaneous absorption study *In vivo* GLP compliance not specified (study considered reliable with restrictions) - 5 human volunteers (involved in another study 2 years before) - Dermal exposure by placing four fingers of the left hand into a vessel filled with pure liquid solvent - Exposure conc.: 100% - Exposure duration: 2 h Measurement of skin thickness and finger volume at regular intervals. Blood and urine analysis were performed during a 24 h period after the beginning of exposure.	No irritation of skin of exposed fingers, but appeared more rigid, wrinkled and less elastic after exposure (max. 2 – 4 h after exposure, then effects gradually disappeared). Volume of the fingers and skin thickness decreased and then return to the normal. 2-butoxyethanol detected in the blood of all subjects after exposure. Estimated skin uptake: great interindividual variation: 7 - 96 nmol/min/cm^2 (0.05 - 0.63 mg/cm^2/hr); median: 20 nmol/min/cm^2 (0.14 mg/cm2/hr). $T_{1/2}$ for 2-butoxyethanol in blood: 0.6 - 4.8 h (mean: 1.3 h). Excretion rate of BAA in urine increased during the first hours of exposure (max. 5 h after exposure start). Cumulative excretion of BAA: 2.5 - 39 % of 2-butoxyethanol uptake (mean: 17 %).	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Johanson et al. (1988)
Percutaneous absorption study *In vivo* No TG followed. Not GLP compliant. (study considered reliable with restrictions) - 4 male human volunteers - Inhalative exposure (mouth only) - Exposure conc.: 50 ppm (2 nmol/m^3) - Exposure duration (inhalative): 2 h - 1 h recovery period - Subsequent percutaneous exposure to vapour in an exposure chamber (naked exposure [exposed surface	Inhalative exposure: - 2-butoxyethanol in blood increased during first h, then steady state at about 3µM (1.8-4µM) during 2nd h. - Mean respiratory uptake: 1.3 mmol or 11 µmol/min - Blood clearance: 3.8 L/min. Percutaneous exposure: - 2-butoxyethanol in blood increased to about 9 µM during 2nd h of exposure. - Average 2-butoxyethanol blood concentration 2.4 - 5.5 times higher after skin exposure than after inhalation. - High percutaneous absorption of 31 (8.6 - 48) µmol/min (2.5 - 5.9 times higher than respiratory uptake)	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Johanson and Boman (1991)

Method	Results	Remarks	Reference
area: ~16000 cm^2], but breathing of normal air) - Exposure duration (dermal): 2 h - 2 experiments/volunteer >2 weeks apart: 1: 23°C, 19 % relative humidity; 2: 33°C, 71 % relative humidity Monitoring of heart rate during exposure day. Collection of capillary blood samples.	- Half-life of 2-butoxyethanol in blood after percutaneous exposure: 34 min (19 - 53 min). Hot/humid versus cool/dry conditions: - Slightly raised 2-butoxyethanol concentration in blood during mouth and skin exposure under hot and humid conditions.		
Percutaneous absorption study *In vivo* - 6 human volunteers - percutaneous exposure to vapour in an exposure chamber (arm only) - Exposure dose/conc.: 50 ppm to ($13C_2$) 2-butoxyethanol - Exposure duration: 2 h Collection of blood samples from unexposed arm vein for analysis of both 2-butoxyethanol and its major metabolite BAA. Collection of finger prickblood samples from exposed arm only at the end of the 2 h exposure. Blood samples were obtained before exposure and at 10, 20, 30, 40, 60 min and 1.5, 2, 2.25, 2.5, 3, 3.5, 4, 8, 12, 16 and 24 h after exposure initiation. Collection of urine samples before exposure and at 0.12 and 12 - 24 h intervals following exposure initiation for metabolite analysis.	Blood: - No 2-butoxyethanol and BAA in blood samples from the unexposed arm until 30 minutes. - By 1.5 h 2-butoxyethanol detectable in all 6 subjects. - An apparent steady state was reached for 2-butoxyethanol after 1.5 - 2 h. - 2-butoxyethanol rapidly cleared from the blood. Elimination $T_{1/2}$: 0.66 h (in 2 of the 6 subjects). - BAA detectable in all 6 subjects by 1 h. Peak blood concentrations: 3 - 4 h after exposure start. - BAA was less rapidly cleared from the blood than 2-butoxyethanol. Elimination $T_{1/2}$: 3.27 h. - 1500x higher concentration in finger prick blood than the corresponding blood sample taken from the unexposed arm (local absorption). Urine: - No free 2-butoxyethanol detectable - No EG nor glycolic acid detectable - BAA eliminated during the first 12h collection interval. - 2/3 of total amount of BAA excreted in the form of an acid-labile conjugate. In this study the skin permeability coefficient was estimated to be 3 cm/hr.	2-butoxyethanol (CAS-No.: 111-76-2) (purity: 99.6 %)	Corley et al. (1997)
Percutaneous absorption study *In vivo* - 4 human volunteers (2 males, 2 females) - Exposure regimen: exposure on 9 separate occasions, separated by at least 3 weeks: 2 exposures whole body (at 25° C, 40 %); 2 skin only (at 25° C, 40	Mean dermal absorption for baseline conditions: 11 % of the total body burden. High temperature increased dermal absorption significantly (14 %). High humidity increased the dermal absorption but not significantly. Clothing has also little effect on dermal absorption.	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Jones and Cocker (2003)

Method	Results	Remarks	Reference
%); other exposures: skin only with one parameter changed: humidity (60 % and 65 %); low vs. high temperature (20 vs. 30° C); minimal clothing (shorts (and bra) vs. overalls (all-in-one boiler suit); and one industrial scenario (overalls at 30° C and 60 % relative humidity). - Exposure conc.: 50 ppm - Exposure duration: 2h - Other Collection of urine before exposure and after each exposure at 0, 4, 6, 8, 10, 12, 22, 26, 30 and 34 h. Determination of total BAA in urine. Physiological monitoring. The results obtained for skin only exposure expressed as percentage of the whole body measurement.	In the industrial scenario skin absorption is significantly increased compared to baseline conditions (maximum dermal absorption contributes for 42 % of the total body burden [mean 39 %]). Conclusions: Temperature is an important factor to take into account for assessing the percutaneous absorption of 2-butoxyethanol. The use of protective equipment under high temperature and relative humidity can lead to a higher dermal absorption than without protective equipment.		
Percutaneous absorption study *In vivo* - 6 male human volunteers - Dermal exposure on the forearm (~40 cm^2) - Exposure conc.: 100, 90, and 50 % (aqueous solutions) - Exposure duration: 4 h - Inhalative exposure on each volunteer served as reference dosage (93 ± 6.8 mg/m3 for 30 min) Determination of dermal absorption parameters during 24 h after exposure start by measuring excretion of total BAA (free + conjugated) in urine and 2-butoxyethanol in blood. Collection of blood samples for 8 h (16 samples per experiment). Collection of urine samples every 4 h during a 24 h period. Each volunteer was exposed twice to a 50 % 2-BUTOXYETHANOL concentration (on each arm), once to the 90 % concentration and once to the pure 2-butoxyethanol. The period between two dermal exposures of the same site was at least 4 weeks.	No 2-butoxyethanol detectable in blood after exposure to pure 2-butoxyethanol. Mean dermal flux and the permeability coefficients were greater for the 50 % dilution than for the 90 % dilution. The same results were obtained with urinary excretion of BAA. Permeation rate reach plateau 1 - 2 h after exposure start (steady state permeation). Comparison of dermal and inhalative uptake: significant amounts of 2-butoxyethanol due to dermal exposure. Half-life of BAA: 3.4 h (1.3 - 3.8 h for inhalation experiment). 57 % of the inhaled 2-butoxyethanol excreted as BAA in urine.	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Jakasa et al. (2004)

Method	Results	Remarks	Reference
Percutaneous absorption study *In vivo* - 4 human volunteers - Dermal exposure - Exposure conc.: 90 % and 50 % aqueous solutions (v/v) - Exposure duration: 2x 4.5 h Sample collection at 30 min intervals throughout the experiment. Determination of percutaneous absorption kinetics in the dialysate samples. The systemic absorption, which is needed to determine recovery of 2-butoxyethanol in the dialysate, estimated from concentration of the main metabolite, free BAA in urine collected at 4 and 4.5 h.	Pseudo steady-state percutaneous absorption at 2 h of exposure for both concentrations. Max. dermal flux of 50% 2-butoxyethanol: 2.8±0.4 mg/cm^2/h. Max. dermal flux of 90% 2-butoxyethanol: 1.9±0.6 mg/cm^2/h. Lag time of 50% 2-butoxyethanol: 25 min. Lag time of 90% 2-butoxyethanol: 39 min. BAA amount ranged from 0.03% to 1.9% of the administered dose of 2-butoxyethanol.	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Korinth et al. (2007)
Other studies			
Kinetic study *In vitro* No TG followed GLP compliance not specified (study not assignable) Determination of degree to which 2-butoxyethanol acts as substrate for alcohol dehydrogenase (ADH) Method not specified - 4 concentrations of 2-butoxyethanol used to define the kinetic constants - Tests at pH 8.8 and 7.4 to determine if pH is a critical factor	Assays conducted at pH 7.4 resulted in data sets which were not suitable for plotting. 2-butoxyethanol: Vmax = 4.06 µM/min Km = 1.18 x 10^{-3} M (correlation coefficient: 0.98).	2-butoxyethanol (CAS-No.: 111-76-2) (purity: 98.6 %)	Dow (1983)
Kinetic study *In vitro* No TG followed GLP compliance not specified (study considered reliable with restrictions) - Perfused rat liver system (Sprague-Dawley) - Tests with and without EtOH - Exposure doses/conc. ranging from 0.057 to 2.7 mM - Exposure duration: 10 min	- Elimination according to Michaelis-Menten equation - Max. elimination rate: 0.59 - 1.3 µmol/min/g - Estimated Km: 0.19 - 0.4 mM - Max. clearance: 2.7 - 3.1 ml/min/g - EtOH decreased extraction ratio from 0.44 to 0.11 - After EtOH withdrawal, liver returned to previous elimination capacity in approximately 10 min Conclusions: 2-butoxyethanol is mainly metabolised by ADH in the rat liver.	2-butoxyethanol (CAS-No.: 111-76-2) (purity: 99 %)	Johanson et al. (1986b)

Method	Results	Remarks	Reference
Collection of 2 samples from perfusion medium and from perfusate during the last 4 min. Determination of 2-butoxyethanol Concentration. At the end of 3 experiments, the effect of EtOH was studied while maintaining the concentration of 2-butoxyethanol at 0.45 mM. Liver was then perfused with a medium containing 17.1 mM EtOH during 20 min, followed by an equal period of time of perfusion with EtOH free medium.			
Kinetic study *In vitro* No TG followed GLP compliance not specified (study not assignable) - SD rats and Syrian Golden Hamster - Assessment of testicular and hepatic capacities to metabolise 2-butoxyethanol by alcohol dehydrogenase (ADH) Method not specified.	Testicular and hepatic activities of ADH for 2-butoxyethanol greater in hamsters than in rats.	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Moslen et al. (1995)
Metabolic study *In vitro* No TG followed GLP compliance not specified (study considered reliable with restrictions) - Human from organ transplant donors, 4 males, 3 females, ages 15-36yrs and rat (Fisher 344) hepatocyte cultures - Incubation doses: 0.02, 0.2, 2 and 10 mM - Incubation period: 4 h - Reverse phase HPLC used to separate metabolites.	90 % of the radioactivity as 2-butoxyethanol, BAA and EG. BAA major metabolite in both species. > 90 % of 0.2 mM 2-butoxyethanol rapidly converted to BAA in rat hepatocytes, 40 % in human hepatocytes. Similar % in both species after 4 h at 0.02 – 0.2 and 2 mM. Human hepatocytes: less 2-butoxyethanol metabolised to BAA at 2 mM compared to 0.2 mM. Highest metabolisation rate of 2-butoxyethanol to EG at lowest substrate concentration (in humans and rats). Higher Vmax values (15–20 fold) in rat hepatocytes than human hepatocytes (741 nmol/h/106 hepatocytes in rats vs. 113 nmol/h/106 in humans). Similar Km in rats and humans (1 mM).	2-butoxyethanol (CAS-No.: 111-76-2) (purity unknown)	Green et al. (1996)
Modelling study Development of PBPK model with the results obtained from various	Good agreement between simulated and experimental blood concentration curves, indicating that assumptions made have a certain degree of validity.	2-butoxyethanol (CAS-No.: 111-76-2)	Johanson (1986)

Method	Results	Remarks	Reference
studies. Concentration-time curves generated by computer simulation. Comparison of simulation outcome to results from experimental exposure studies in male human volunteers. Assumptions: solvent uptake only in lungs, elimination only in liver; distribution of solvent instantaneously and homogenously in each compartment (solvent retained in the respiratory airways immediately reaches arterial blood).	Increased physical activity increased 2-butoxyethanol blood concentration (due to increased pulmonary uptake rate). Co-exposure to EtOH caused elevated 2-butoxyethanol blood concentration (due to decreased elimination rate). Rapid decay of 2-butoxyethanol. Low risk of accumulation of unmetabolised solvent in the body. Non-linearities due to saturated elimination occur at concentrations above 100 ppm, even in combination with physical exercise and EtOH. Thus linear kinetics of 2-butoxyethanol expected at ordinary occupational inhalation exposure.		
Modelling study (improvement of the existing model by Johanson 1986 c) - Modelling of metabolite formation by three routes of exposure (oral, dermal, inhalation) in rats and humans - Incorporation of the BAA disposition into the model - Addition of allometric scaling factors for rat and human physiological parameters - Addition of competing pathways for the metabolism of 2-butoxyethanol into the model.	*In vivo* data generally in good agreement with the model except for dose levels which cause toxicity. No systematic provision of the model for correcting for potential diminished renal excretion and/or liver metabolism that arise secondary to the haemolytic activity of BAA. Therefore overprediction of BAA amounts excreted by kidneys via urine possible. Model satisfactorily predicts BAA concentrations in blood and BAA elimination via urine at dose levels that do not cause toxicity.	2-butoxyethanol (CAS-No.: 111-76-2)	Shyr et al. (1993) and Dow (1993) and Corley et al. (1994) and Lee et al. (1998)

8.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Animal studies

Summary oral route:

To examine the kinetic properties of 2-butoxyethanol by oral application 11 studies are available, 7 performed using Fischer 344 rats, one performed with Sprague Dawley rats and three performed using B6C2F1 mice. The studies demonstrated that absorption of orally administered 2-butoxyethanol was rapid and essentially complete (assumed to be 100 %). Specific target organs were the forestomach (especially in mice), the liver and the kidneys.

In rats and mice the major metabolite of 2-butoxyethanol was shown to be butoxyacetic acid (BAA), which is formed by a mechanism involving alcohol and aldehyde dehydrogenases (ADH). Simultaneous administration of 2-butoxyethanol and a primary alcohol (ethanol, n-propanol or n-butanol) in sufficient quantity inhibited BAA formation. The other metabolites were (in order of magnitude): the glucuronide conjugate of 2-butoxyethanol (BEG; percentage increases relative to the dose at the expense of BAA formation) and two minor metabolites, the sulphate conjugate of 2-butoxyethanol and ethylene glycol (BES and EG, respectively).

Elimination of 2-butoxyethanol was shown to be rapid and excretion occurred mainly via urine in rats and mice, however, the elimination rate was slightly higher in rats compared to mice (59 % versus 48 % of total dose excreted via urine during the first 24 h). Here, an inverse dose-relationship was observed: in rats treated with a lower dose of the test substance, a higher urinary excretion was measured than in rats treated with a higher test dose (70 % at 125 mg/kg bw/day versus 40 % at 500 mg/kg bw/day). Some metabolised 2-butoxyethanol was eliminated as CO_2 in expired air (0 – 20 % for a high and a low dose, respectively), while only a small amount of unmetabolised 2-butoxyethanol (approximately 1 %) was eliminated in expired air. 2 – 3 % of 2-butoxyethanol was excreted via faeces. In two studies, BAA, BEG and 2-butoxyethanol were found in bile. 2-butoxyethanol was shown to not accumulate in tissues.

The metabolic profile of 2-butoxyethanol after repeated exposures was similar to the profile obtained after a single, acute exposure. However, age-related differences were observed: young rats eliminated 2-butoxyethanol and its metabolites to a greater extent than adult rats, especially via CO_2 and urine. Moreover, young rats excreted significantly less BAA but more BEG compared to older rats.

Summary inhalation route:

To assess the kinetic properties of 2-butoxyethanol after inhalation, three studies were evaluated with rats (two with Fischer 344 and one with Sprague Dawley rats) and further three studies with B6C3F1 mice.

One of the studies assessed the distribution of 2-butoxyethanol and its metabolites over time (Green et al., 2000). This study clearly showed that the liver, blood, buccal cavity and the forestomach are main target organs, in which the highest concentrations of the test substance and especially the metabolites of 2-butoxyethanol could be detected. Great amount of radiolabelling that was found in the gastro-intestinal tract was demonstrated to be caused by ingestion during grooming behaviour and/or by mucociliary clearance from nasopharynx, but not directly by inhalation of the substance. The great amounts of radiolabelling found in the forestomach, on the other hand, were suggested to be the result of systemic distribution after 2-butoxyethanol inhalation.

As via oral exposure, inhaled 2-butoxyethanol was metabolised mainly to BAA, EG and BEG. However, two further unidentified metabolites were detected in small quantities. BAA and EG formation followed a saturable mechanism. Increased doses of 2-butoxyethanol led to an increased formation of BEG compared to BAA and EG.

As seen after oral exposure, elimination by urinary excretion was rapid and predominant. A small amount of the administrated dose was again eliminated as CO_2 (less than 10 %).

The blood half-life of 2-butoxyethanol was determined to be about 10 minutes in rats and approx. 5 minutes in mice, independently of the exposure concentration.

While BAA seemed to be eliminated by a saturable, non-linear mechanism, elimination of 2-butoxyethanol followed a linear kinetic. When repeated doses of 2-butoxyethanol were administered, the rate of BAA elimination tended to decrease, especially when 2-butoxyethanol concentrations were high. Moreover, a slower elimination rate of 2-butoxyethanol was demonstrated with prolonged exposure.

A species difference in elimination of 2-butoxyethanol was reported with mice eliminating 2-butoxyethanol 2-fold faster than rats. In rats, moreover, elimination of BAA varied with sex. Females tended to eliminate BAA slower than males. This difference between the sexes could be attributed to

general differences in renal excretion between male and female rats, whereas in mice, such a sex difference could not be found. Furthermore, similar to the age-related findings in rats after oral exposure, an age difference in elimination of 2-butoxyethanol was described in mice after inhalative exposure to the test substance. Older mice had a 10-fold lower BAA blood concentration after 24 h than younger mice. However, after continuous exposure, age-differences disappeared.

Summary dermal route:

Six studies are available to assess the kinetic properties of 2-butoxyethanol after dermal application of the test substance. Four studies were performed using rats (one with Sprague-Dawley rats, two with Wistar rats and one with Fischer 344 rats) and two were performed using guinea pigs. 2 further *in vitro* studies are available, examining the percutaneous uptake of 2-butoxyethanol in the skin of various species, at different concentrations using different solvents. The test substance was either applied semi-occlusively or occlusively.

The relevant studies demonstrated that under semi-occlusive conditions, dermal uptake rates of pure 2-butoxyethanol was between 20 and 30 % of the administrated dose. The dermal uptake of aqueous dilutions of 5, 10 and 20 % 2-butoxyethanol was similar to that of the pure substance, while the uptake was significantly increased almost 2-fold for 40 and 80 % aqueous solutions of 2-butoxyethanol. The rate of penetration under occlusive conditions, on the other hand, was less for the pure substance compared to a 50 % aqueous solution, potentially due to the volatility of 2-butoxyethanol. In one study, between 43 and 64 % of the dermally applied dose was trapped as volatile $_{14}C$.

In an *in vitro* study, it was demonstrated that the dermal uptake for pig skin was 2 - 3 times slower than for rat skin. It was further shown that the penetration rate for human skin is comparable to that of pig skin. Furthermore, it was indicated that absorption of 2-butoxyethanol *in vitro* through rat skin most closely reflected the penetration *in vivo.*

It was also shown that approximately 2 h after the beginning of the respective dose application, a peak in plasma 2-butoxyethanol and/or BAA was obtained, which stayed relatively constant until the end of exposure. Metabolism of 2-butoxyethanol mainly led to the formation of BAA and only small quantities of BEG, as demonstrated during oral and inhalative exposure. The half-life of metabolites in plasma was about 4 h. Again, the majority of metabolites were eliminated via urinary excretion and only a very small amount was found in faeces. Furthermore, a small part of administered 2-butoxyethanol was metabolised and exhaled as CO_2. The amount of exhaled 2-butoxyethanol in form of CO_2 increased with increasing exposure dose.

Summary other routes:

There are further studies available applying other, non-physiological routes of application, such as intravenous (IV), intraperitoneal (IP) or subcutaneous (SC) applications. Two studies are available assessing the toxicokinetics of 2-butoxyethanol via IV route (one in rats, one in mice), while three studies were evaluated using the IP route (one in rats, two in mice) and two applying the SC route (one in rats, one in mice). Target organs evidenced in the previous described studies were confirmed in these studies: spleen, liver, (thymus) and stomach.

As in the inhalation studies, slight differences were seen in the distribution of the substance between the forestomach and the glandular stomach, especially after an IV injection in mice (rats not tested). The distribution within the stomach resulted from systemic circulation and also from ingestion of 2-butoxyethanol (which could come from salivary glands), whereas the accumulation in the forestomach was again suggested to be the result of systemic circulation of 2-butoxyethanol only.

In these studies, it was again demonstrated that BAA is the major metabolite in both, rats and mice. It was further shown that the formation of BAA was caused by mechanisms involving alcohol and aldehyde dehydrogenases in the liver and that the renal organic acid transport might play a role in the clearance of BAA.

Furthermore, similar to the age-related findings in rats after oral and inhalative exposure, an effect of age was described for C_{max} and area under the curve (AUC) of 2-butoxyethanol and BAA after IV exposure of rats to the test substance, with adults showing a higher sensitivity than young animals. However, no effect of age was detected regarding the half-life of 2-butoxyethanol.

Human data

Summary humans, inhalation route:

Five studies are available in which human volunteers were exposed to 2-butoxyethanol by inhalation. One further study examined the effect of an incidental, occupational exposure of workers of a beverage packing production to 2-butoxyethanol (co-exposure to methyl ethyl ketone).

Results suggest that due to a "wash in/ wash out" mechanism of the respiratory tract the hydrophilic 2-butoxyethanol is adsorbed to the surface of the respiratory tract during inspiration and desorbed during exhalation leading to a decrease in the real uptake of substance. Further results show similar patterns as obtained by animal experiments: Rapid uptake of 2-butoxyethanol with peaks in plasma concentrations after approx. 2 hours, followed by decay. The blood half-life of 2-butoxyethanol with 40 minutes was higher than in rats (10 minutes) and mice (5 minutes) after inhalation. The main metabolite was - as in the other mammal species tested – BAA and most of the test dose and BAA was also excreted via urine.

Summary humans, dermal route:

Six *in vivo* studies on human volunteers and six *in vitro* studies are available for this route of exposure. The *in vitro* studies examined among others the rate of absorption of liquid 2-butoxyethanol through human skin. The obtained results, however, vary by a factor of 25 (0.064 mg/cm^2/hr vs. 1.66 mg/cm^2/h). *In vitro*, furthermore, the rate of absorption was highly dependent on the concentration of the aqueous solution of 2-butoxyethanol used. *In vivo*, the interindividual variation was also very high: one study calculated an estimation of the skin penetration of 7 – 96 nmol/min/cm2 (0.05 mg/cm^2/hr - 0.63 mg/cm^2/hr) for pure liquid 2-butoxyethanol. Another study performed with liquid 2-butoxyethanol demonstrated that absorption is greater if a 50 % aqueous solution of 2-butoxyethanol is used compared to neat 2-butoxyethanol, similar as it was demonstrated in rodents after occlusive dermal exposure.

Again, as in the animal studies, a peak in 2-butoxyethanol in plasma was found after approx. 2 hours, followed by a rapid decrease. Most of the substance and its metabolites were also eliminated by urinary excretion. BAA was once more the major metabolite. The blood half-life of 2-butoxyethanol with approximately 1 h was again higher than in mice and rats after inhalation of the test substance.

The studies moreover showed that increasing temperature and humidity increased the percutaneous uptake of 2-butoxyethanol vapour and that wearing protective clothing equipment is counterproductive as it leads to an even higher dermal absorption rates as without wearing protective equipment when temperature and humidity are high.

Summary other data:

In vitro studies have shown that 2-butoxyethanol transformation to BAA is depended on ADH in rat liver. This enzyme seems to be more active in females than in males.

Moreover, it has been demonstrated *in vitro* that ~ 90 % of 2-butoxyethanol is rapidly converted to BAA in rat hepatocytes, whereas only ~ 40 % of the test substance is converted to BAA in human hepatocytes within the same time. Moreover, the higher the exposure concentration (0.2 mM vs. 2 mM) the less 2-butoxyethanol is converted to BAA in human hepatocytes. The metabolic rate (V_{max}) in rats was shown to be 10 – 20-folds higher than in humans.

A PBPK model was developed using experimental data collected in humans and in animals. This model included various exposure routes (inhalation, oral, dermal, IV), accounted for differences between humans and animals, implicated kinetic parameters of the main metabolite BAA and allowed for the modelling of repeated exposures. Results of recent studies seem to be comparable and are consistent with the current PBPK model. Hence the model seems to facilitate toxicokinetic extrapolation between animals and humans. The model, for instance, predicted that 2-butoxyethanol is metabolised and eliminated faster (per kg bw) in rats compared to humans, as it was demonstrated in *in vivo* studies, which reported a longer blood half-life of 2-butoxyethanol in humans than in rats (and mice) after inhalation. Accordingly, the model predicted that exposure to 2-butoxyethanol results in higher peak BAA blood concentrations in rats versus humans within a restricted experimental period. Furthermore, an assessment factor for interspecies differences of 7.2 (allometric scaling) was estimated using the PBPK model, assumedly accounting for toxicokinetic effects. However, it needs to be kept in mind that the model is partly based on assumptions made from human studies using low numbers of subjects ($n \leq 7$), and which moreover showed very high interindividual variation. Thus, although reviews found the model to be of reasonable quality with capabilities to simulate many relevant pharmacokinetics data sets, model limitations were also stated and therefore critical analysis of model predictions, including variability, uncertainty, and sensitivity is essential (Meek et al., 2013).

Taken together, results in humans are comparable to those obtained with other mammals (e.g. similar time profiles in blood and urine, same metabolites, same excretion routes). Although humans seem to be comparatively less sensitive to exposure to 2-butoxyethanol than rats (e.g. slower percutaneous absorption *in vitro* compared to rat skin, lower susceptibility of erythrocytes to adverse BAA effects *in vitro* compared to rat erythrocytes), the blood half-life of 2-butoxyethanol was shown to be longer in humans than rats and, furthermore, the interindividual variation among humans was eminently high.

9 EVALUATION OF HEALTH HAZARDS

Acute toxicity

9.1 Acute toxicity - oral route

Table 9: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
LD_{50}-Test, no guideline followed	**Rat**, Wistar, male (90-120 g), 10/group	2-butoxyethanol (CAS: 111-76-2) (purity: commercial	1150 to 1910 mg/kg bw, single dose (stomach tube), 14d post exposure observation period	1480 mg/kg bw (male) Calculated by probit method	Smyth et al. (1941)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
		grade)		Death within 2d after dosing	
LD_{50}-Test, similar to OECD TG 401	**Rat**; Wistar (until 1942), then Sherman (1942-1952), Carworth-Wistar (from 1952); males and females; 10/group	2-butoxyethanol (CAS: 111-76-2)	1150-3700 mg/kg bw, single dose, 14d post exposure observation period	560-2800 mg/kg bw (males) 530-2300 mg/kg bw (females) Sluggishness, ruffling of coats, prostration, narcosis	Carpenter et al. (1956) and Mellon Institute of Industrial Research (1952)
LD_{50}-Test, similar to OECD TG 401	**Rat**, strain not specified, female (150-200 g), 5/group	2-butoxyethanol (CAS: 111-76-2)	252-1000 mg/kg bw, single dose, mortality in 3/5 at 500 mg/kg bw, showing haematuria	470 mg/kg bw (calculated)	Dow (1959)
LD_{50}-Test, similar to OECD TG 401	**Rat**, strain not specified, females, 10/group	2-butoxyethanol (CAS: 111-76-2)	1000-4000 mg/kg bw single dose, 7d post exposure observation period mortality from 1600 mg/kg bw	1950 mg/kg bw (calculated)	Hoechst A. (1966)
LD_{50}-Test, similar to OECD TG 401	**Rat**, Wistar, males, 10/group	Polysolv EB (2-butoxyethanol, purity unknown)	670-5000 mg/kg bw single dose, 14d post exposure observation period; mortality: 670 mg/kg bw: 0/10; 1310 mg/kg bw: 3/10, 2560 mg/kg bw: 9/10; 5000 mg/kg bw: 10/10 Lethargy, laboured breathing, haemolysis, liver and kidney toxicity; mortality from 1310 mg/kg bw	1590 mg/kg bw (calculated)	MB research laboratories (1976)
LD_{50}-Test, similar to OECD TG 401	**Rat**, Wistar, males, 5/group	2-butoxyethanol (CAS: 111-76-2)	9030-1128 mg/kg bw single dose, 14d post exposure observation period; mortality: 9030 mg/kg bw: 5/5 4515 mg/kg bw: 5/5 2257 mg/kg bw: 2/5 1128 mg/kg bw: 0/5 Laboured breathing, sluggish and bloody salivation; Haemolysis, dark liver and red kidneys.	1670-3504 mg/kg bw 2420 mg/kg bw (calculated)	Bushy Run Research Center (1980b)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
No details of the study are given	**Rat**, CDF, females, 3/group	2-butoxyethanol (CAS: 111-76-2) undiluted (purity unknown)	130–2000 mg/kg bw 2000 mg/kg bw: 2/3 Lethargy, laboured breathing, necrosis of the tail; Mortality from 2000 mg/kg bw	1000-2000 mg/kg bw	Dow Chemical Co. (1981)
No details of the study are given	**Rat**, CD/BR, males, in fasted and fed rats 5/group	2-butoxyethanol (CAS: 111-76-2) (purity: > 99.5 %)	five different doses progressing by a factor of 2 Inactivity, laboured breathing, anorexia, tremors, haemolysis	1746 mg/kg bw	Eastman Kodak (1981a)
No details of the study are given	**Rat**, strain and number tested not given	2-butoxyethanol (CAS: 111-76-2)	No data	620 mg/kg bw calculated	Rowe and Wolf (1982)
LD_{50}-Test, similar to OECD TG 401	**Mouse**, strain and number tested not given, males (20-30 g)	2-butoxyethanol (CAS: 111-76-2) (commercial grade)	940-1620 mg/kg bw	1230 mg/kg bw	Carpenter et al. (1956)
No details of the study are given	**Mouse,** CD1, males, in fasted and fed mice, 5/group	2-butoxyethanol (CAS: 111-76-2) (purity: > 99.5 %)	five different doses progressing by a factor of 2 Laboured breathing, anorexia, tremors, haemolysis	1519 mg/kg bw (fasted mice) 2005 mg/kg bw (fed mice)	Eastman Kodak (1981a)
No details of the study are given	**Mouse**, strain and number tested not given	2-butoxyethanol (CAS: 111-76-2)	No data	1170 mg/kg bw when fed as a water solution; 1700 mg/kg bw when fed as an oil solution	Rowe and Wolf (1982)
No details of the study are given	**Mouse**, strain and number tested not given	2-butoxyethanol (CAS: 111-76-2)	No data	1000-1600 mg/kg bw	Saparmamedov (1974)
LD_{50}-Test, similar to OECD TG 401	**Rabbit**, strain and number tested not given, males (a) 1500-3000 g, (b) 2700-3200 g	2-butoxyethanol (CAS: 111-76-2)	No data	(a) 320 mg/kg bw (b)370 mg/kg bw	Carpenter et al. (1956) and Tyler (1984)
LD_{50}-Test, no guideline followed	**Guinea pig** (250-300 g), 10/sex/group	2-butoxyethanol (CAS: 111-76-2) (purity: commercial grade)	960-1500 mg/kg bw	1200 mg/kg bw Calculated by probit method	Smyth et al. (1941) and Carpenter et al. (1956)
LD_{50}-Test (gavage),	**Guinea pig**,	2-butoxyethanol	500-2000 mg/kg bw	1414 mg/kg	Eastman

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD TG 401	Hartley strain (5-7 wk of age), males and females, 5/sex/group	(CAS: 111-76-2) (purity: 99.87 %)	mortality: 500 mg/kg bw: male/female: 0/5 1000 mg/kg bw: male/female: 1/5 2000 mg/kg bw: males: 3/5, females: 5/5 Weakness, prostration, necrosis and haemorrhage of gastric mucosa; Mortality from 1000 mg/kg bw	bw (calculated for both sexes with a 95 % confidence level of 1020 to 1961 mg/kg bw)	Kodak (1994b) cited in Gingell et al. (1998)

Table 10: Summary table of human data on acute oral toxicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Case report	12 % of 2-butoxyethanol, corresponding to about 0.5 to 1 g/kg bw	Suicide attempt of a 50-year woman ingested 250-500 ml of a window cleaner containing 12 % of 2-butoxyethanol	On admission to the intensive care unit, the patient was comatose. Biochemically she presented with metabolic acidosis, hypokalaemia, a rise in serum creatinine level and a markedly increased urinary excretion of oxalate crystals.	Rambourg-Schepens et al. (1988)
Case report	Mixture containing 12.7 % of 2-butoxyethanol and ethanol (3.2 %) (about 57 g of 2-butoxyethanol, corresponding to about 1 g/kg bw	Suicide attempt of a 23-year woman (weighing 64 kg with a psychiatric history), ingested about 500 mL of a mixture containing 2-butoxyethanol and of EtOH	Coma, hypotension, breathing difficulties and metabolic acidosis; 432 mg/L 2-butoxyethanol in the blood (upon admission), 304 mg/L (2 hr after admission); Haematuria and decreased Hb concentration (11.9 g/dL on admission to 8.9 g/dL on the second day for 2 d).	Gijsenbergh et al. (1989)
Case report	Mixture containing 9.1 % 2-butoxyethanol (45.5 g) and ethanol (2.5 %), corresponding to about 750 mg/kg bw	Acute poisoning of a 53-year man (chronic alcohol abuser) ingested about 500 mL of a household cleaning fluid, a mixture containing 2-butoxyethanol and ethanol	Coma, tachycardia, metabolic acidosis, hypoxemia, pulmonary oedema and ARDS (Adult Respiratory Distress Syndrome), 36 hr after admission non haemolytic anaemia with thrombopenia	Bauer et al. (1992)
Some cases	Mixtures	Report from Pittsburgh Poison Center	All children were	Dean and

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
of children poisoning reported from Poison Center (24 pediatric patients	containing 2-butoxyethanol in concentrations ranging from 0.5 % to 9.9 %	of cases of children poisoning, ages ranged from 7 months to 9 years, ingested quantities ranged from 5 to 300 mL of a liquid glass cleaner, 2 of the 24 children ingested > 15 mL and were treated by gastric emptying and 24 h hospital observation	asymptomatic immediately following the ingestion.	Krenzelok (1992)
Case report	Mixture containing 22 % 2-butoxyethanol (maximum 95 g), corresponding to about 1.25 g/kg bw	18-year man ingested 360 mL and 480 mL of a glass cleaner containing 2-butoxyethanol, on two separate occasions by 9 days	10 hrs after the first ingestion: hospitalised with severe CNS depression, metabolic acidosis, haematuria, and hepatic biochemical disorders (SGOT, SGPT, hepatic bilirubin); then 10 d after: nothing after the second ingestion; recovered on both occasions without sequelae	Gualtieri et al. (1995) and Gualtieri et al. (2003)
Case report	Product (alkaline corrosive, pH 13) containing 25-35 % 2-butoxyethanol (maximum 336 g), corresponding to about 4.5 g/kg bw	19-year man ingested about 20-30 ounces of 'Spitfire', a product containing 2-butoxyethanol (and further propylene glycol 15-25 %, monoethanolamine 5-10 %, and potassium hydroxide 1-3 %)	Coma, acidosis and haematuria, exhibited neurologic sequelae (difficulties with fine motor skills and stop speaking)	Burkhart and Donovan (1998)
Case report	Mixture containing 10-30 % 2-butoxyethanol and 10-40 % isopropanol (estimate dose of 24-72g), corresponding to a range of 0.4–1.2 g/kg bw	Suicide attempt of a 51-year woman ingested 8 ounces of a 'Sanford Expo White Board Cleaner, a mixture containing 2-butoxyethanol and isopropanol	prolonged hyperchloremic metabolic acidosis and mental status depression; she recovered without apparent sequelae	McKinney et al. (2000)

Table 11: Summary table of other studies relevant for acute oral toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
LD_{50}-Test	2-butoxyethanol (CAS: 111-76-2)	**Rat** Single i.v. injection of a 3 % dilution in 0.75 % NaCl in females (170-230 g) and undiluted in females (90-120 g)	LD_{50} of a 3 % dilution = 380 mg/kg bw (290–500 mg/kg bw) LD_{50} (undiluted) = 340 mg/kg bw (300 to 380 mg/kg bw)	(Mellon Institute of Industrial Research, 1952) and Carpenter et al. (1956)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
LD_{50}-Test	2-butoxyethanol (CAS: 111-76-2)	**Rat** Single i.p. injection of 2 compounds with different purities (n butyl oxitol, 98-99 %; Dowanol EB, >99 %) in female SD rats; doses of 200, 252, 316, 398 were tested for both substances with an additional dose of 500 mg/kg bw for Dowanol EB	Mortality was seen from 252 mg/kg bw; in all dose groups and with both substances blood was seen in urine (haemolysis) LD_{50} of n butyl oxitol = 252 mg/kg bw LD_{50} of Dowanol EB = 317 mg/kg bw	Dow (1972)
Test examined kidney function by parameters in urine	2-butoxyethanol (CAS: 111-76-2)	**Rat** Single i.v. injection of 0.034 mL/kg in 10 female SD rats; then over the next 4 days 24-hr urine samples; urinalysis (volume, osmotic pressure (by means of freezing point depression), haematuria (using a semi-quantitative test), albumin, Lactate Dehydrogenase (LDH)), gel filtration of the urine was carried out before enzyme and albumin analyses	Disturbances in the whole nephron, indices were: Increase of albumin and LDH activity on the 2nd day and decrease of GAL activity on the 4th day; 2/10 microhaematuria on the 1st day	Freundt and Helm (1986)
Test examined renal changes	2-butoxyethanol (CAS: 111-76-2)	**Rat** Single i.v. injection of 0.034 mL/kg in female SD rats (200 g), 8/group; activities of lactate dehydrogenase (LDH), leucine aminopeptidase (LAP) and beta-galactosidase (GAL), the concentrations of albumin and creatinine, the volume, the specific gravity and the pH, leucocytes, erythrocytes, nitrite, total protein, ketone, bilirubin and urobilinogen were analysed in the 24 hr urine samples	Slight nephrotoxic potential Increase of urinary albumin indicates an impairment of the glomerular region in the kidney which were transient	Freundt et al. (1993)
LD_{50}-Test	2-butoxyethanol (CAS: 111-76-2)	**Mouse** Single i.v. injection of a 3 % dilution in 0.75 % NaCl in male and female mice (15-35 g)	LD_{50} = 1130 mg/kg bw	Carpenter et al. (1956)
LD_{50}-Test	2-butoxyethanol (CAS: 111-76-2)	**Rabbit** Single i.v. injection of a 3 % dilution in 0.75 % NaCl in males (2500-3000 g) and Undiluted in males (2500-3000 g)	$LD_{50\ (dilution)}$ = 500 mg/kg bw (380-650 mg/kg bw) $LD_{50\ (undiluted)}$ = 280 mg/kg bw	Carpenter et al. (1956)

9.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Animal studies

In rats, numerous studies have been performed to assess the LD_{50} via oral route. Results vary a lot between 470 mg/kg bw and 2800 mg/kg bw. Recent studies (performed according to well defined experimental methods) have given results between 1000 and 2600 mg/kg bw. Clinical signs noted were lethargy, laboured breathing, and ataxia. For pathology, haemolysis was seen in the majority of the studies, sometimes accompanied with renal and hepatic lesions (most probably as a consequence of haemolysis).

In mice, available studies exhibited LD_{50} ranging from about 1200 to 1600 mg/kg bw (these results are more consistent than those obtained in rat studies). Clinical symptoms similar to those in the rat studies were seen.

One study was performed in rabbits showing a LD_{50} ranging from 320 to 370 mg/kg bw. This value seems very low compared to other studies on other species via oral route. The rabbit can be considered to be the most sensitive species concerning acute oral toxicity of 2-butoxyethanol.

Two studies are available in Guinea pigs. The LD_{50} calculated were 1414 and 1200 mg/kg bw. The same clinical signs and pathology than other species tested were seen in these studies. Necrosis and haemorrhage of the gastric mucosa was also seen.

Some studies performed via i.v. and i.p. routes in various species gave different results. These studies are not suitable for LD_{50} identification because these routes of administration are not relevant.

Animal experiments have shown that 2-butoxyethanol can cause, in high concentrations and after a variable symptom-free interval, CNS depression, nephrotoxicity, damage to the liver and lung, abnormal blood picture with erythropenia, reticulocytosis, granulocytosis, and an increased fragility of the erythrocytes inducing haemolysis and haemoglobinuria.

Human data

Acute human toxicity data were reported from children accidental ingestion or adult suicide attempts made with mixtures containing 2-butoxyethanol. For case reports, ingested doses are difficult to evaluate because of the lack of data concerning the body weight of all patients and the exact ingested dose, but a semi-quantitative estimation of the ingested doses was made for each case. 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 and metabolic acidosis. Signs of haemolysis were seen in some cases but this finding was not systematic (this showed that human is much more resistant to haemolysis than rodents). After a first acute ingestion, a second administration some days later did not exhibit the same symptoms, and this finding was also seen with animals in some studies. In these cases, 2-butoxyethanol was ingested together with other substances (ethanol and/or unknown substances) that could have some influence on the symptoms seen. Between 0.5 and 1.5 g/kg bw the patients totally recovered after treatment. Overall manifestations of acute 2-butoxyethanol toxicity include metabolic acidosis, haemolysis, hepatorenal dysfunction, and coma, but vary widely in reported cases. A LOAEL of 400 mg/kg bw can be taken into account for acute toxicity by oral route in humans. It should be noted that this is a worst case estimation derived from McKinney et al. (2000) in which the possible range of exposure was between 400 and 1200 mg/kg bw.

9.1.2 Comparison with the CLP criteria

Acute oral toxicity means those adverse effects occurring following administration of a single dose of a substance or a mixture, or multiple doses given within 24 hours. Acute toxicity relates to effects occurring after a single exposure to a substance or mixture. Acute toxicity classification is generally

assigned on the basis of evident lethality. The evidence for acute toxicity of 2-butoxyethanol is obtained from animal testing. A number of human case studies are available from attempted suicides with mixtures containing 2-butoxyethanol. According to these data it is suggested that the human acute toxicity dose level is in the region of 400 mg/kg bw.

Substances can be allocated to one of four toxicity categories based on acute toxicity by the oral route according to the criteria shown in the Table 3.1.1 of Annex I, Part 3, Table 3.1.1 of CLP.

The following applies for the classification as

'Acute oral toxicity - Category 4: 300 < ATE ≤ 2000 mg/kg bw.'

Based on the lowest oral LD_{50}-values in animals (320 mg/kg bw in rabbits, about 470 mg/kg bw in rats, about 1500 mg/kg bw in mice, and 1200 mg/kg bw in guinea pigs) 2-butoxyethanol fulfils the criteria for classification for acute oral toxicity Category 4.

9.1.3 Conclusion on classification and labelling for acute oral toxicity

According to CLP 2-butoxyethanol has to be classified as:

Acute Tox. 4 for oral exposure and labelled with hazard statement H302: Harmful if swallowed.; with the pictogram "GHS07: Exclamation mark", and with the signal word "Warning".

Acute toxicity Estimate (ATE, oral)

According to Annex I, Part 3, Point 3.1.3.6. ('Classification of mixtures based on ingredients of the mixture (Additivity formula)') classification of mixtures is based on the calculated ATE of a mixture. The ATE for a mixture is determined by calculation from the ATE values when data available for all relevant ingredients or when data are not available for all components according to the appropriate additivity formula (s. CLP Annex I, Part 3, and Point 3.1.3.6.1. or 3.1.3.6.2.3).

For the classification for acute oral toxicity of mixtures containing 2-butoxyethanol an ATE value of 500 mg/kg bw is proposed for the calculation with the additivity formula according to Annex I, Part 3, and Point 3.1.3.6.1. or 3.1.3.6.2.3 of the CLP. The ATE value (oral) for 2-butoxyethanol is converted from the acute toxicity point estimate of acute hazard category 4 (see Table 3.1.2 in the CLP Regulation).

Justification for the converted ATE value (oral) of 500 mg/kg bw:

The relevant and acceptable studies exhibited LD_{50} values for 2-butoxyethanol ranging from 320 to 1500 mg/kg bw. From these studies the LD_{50} for 2-butoxyethanol are 320 mg/kg bw in rabbits, about 470 mg/kg bw in rats, about 1500 mg/kg bw in mice, and 1200 mg/kg bw in guinea pigs. The rabbit is considered being the most sensitive species to acute oral toxicity of 2-butoxyethanol among all species tested, with the lowest LD_{50} of 320 mg/kg bw. The mouse is considered being the least sensitive species to acute oral toxicity, with the highest derived LD_{50} of about 1500 mg/kg bw. Based on all these derived LD_{50}-values, 2-butoxyethanol meets the criteria for classification for acute oral toxicity Category 4.

In this case conversion from the experimentally obtained acute toxicity range value (or acute toxicity hazard category) to an acute toxicity point estimate for use in the formulas (CLP Annex I, Part 3, and Point 3.1.3.6.1. or 3.1.3.6.2.3) for the classification of mixtures is applied. According to CLP, Annex I, Table 3.1.2. the converted acute toxicity point estimate for oral administration classified in the hazard Category 4 is 500 mg/kg bw (see Note 1: 'These values are designed to be used in the calculation of a mixture based on its components and do not represent test results.').

Accordingly, the converted ATE value (oral) of 500 mg/kg bw should be used in the respective additivity formula for the classification of mixtures containing 2-butoxyethanol.

9.2 Acute toxicity - dermal route

Table 12: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, purity**	**Dose levels duration of exposure**	**Value LD_{50}**	**Reference**
LD_{50}-Test, no guideline followed	**Rat**, strain and number tested not given	2-butoxyethanol (CAS: 111-76-2) (purity unknown)	1680-3079 mg/kg bw, 4h exposure (occlusive); 14d post exposure observation period	2275 mg/kg bw	Mellon Institute of Industrial Research (1961)
LD_{50}-Test according to OECD TG 402, GLP study	**Rat**, SD, 5/sex	2-butoxyethanol (CAS: 111-76-2) (purity unknown)	Single dermal application (semi-occlusive) 24h exposure, 14d post exposure observation period No irritation, no sign of toxicity	>2000 mg/kg bw for males and females	Safepharm laboratories (1993a)
LD_{50}-Test according to OECD TG 402, GLP study	**Rat**, SD, 5/sex	2-butoxyethanol (CAS: 111-76-2) (purity unknown)	Single dermal application (occlusive), 24h exposure, 14d post exposure observation period Mortality: 1 female 2 days after dosing showing haemorrhagic lungs, dark liver and kidneys, sloughing of the non-glandular epithelium of the stomach and haemorrhage of the small and large intestines; clinical signs were ataxia, pallor of extremities, lethargy, laboured breathing; no signs of irritation	>2000 mg/kg bw for males and females	Safepharm laboratories (1993b)
LD_{50}-Test, no guideline followed	**Guinea pig,** strain and number tested not given	2-butoxyethanol (CAS: 111-76-2) (purity unknown)	5400-7765 mg/kg bw, no more data	6411 mg/kg bw	Mellon Institute of Industrial Research (1952)
LD_{50}-Test, no guideline followed, but according to U.S. Federal Hazardous Substances Labelling Act (21 CFR 191)	**Guinea pig,** Hartley (400-900 g), 4 males/dose	2-butoxyethanol (CAS: 111-76-2) (purity commercial grade, 99.6 %)	3 dosages (undiluted) were tested, single dermal application (occlusive) to intact and abraded skin	230 mg/kg bw (intact skin) 300 mg/kg bw (abraded skin) (calculated by Finney, 1952)	Roudabush et al. (1965)
No guideline followed; a	**Guinea pig**, strain not	2-butoxyethanol (CAS: 111-76-	Single dermal application of different amounts (0.5	≤1800 mg/kg bw	Wahlberg and

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, purity**	**Dose levels duration of exposure**	**Value LD_{50}**	**Reference**
comparative percutaneous toxicity study of 10 industrial solvents	available (352-375 g), 20 animals/group	2) (purity 99 %)	or 2.0 mL, occlusive) for a period of 5 to 7 days (substance totally absorbed); following 35d post exposure observation period Mortality: 450 mg/kg: 0/20, at 1800 mg/kg: 5/20 at Day 3, 11/20 at Day 4, 13/20 at Day 7		Boman (1979)
LD_{50}-Test according to OECD TG 402; GLP study	**Guinea pig,** Hartley, 5 animals/dose	2-butoxyethanol (CAS: 111-76-2) (purity 99.8 %)	2000 mg/kg bw Single dermal application (occlusive), 24 h exposure, 14d post exposure observation period No mortality	>2000 mg/kg bw	Eastman Kodak (1994a)
LD_{50}-Test, similar to OECD TG 402	**Rabbit,** New Zealand White, male, 3-5 months of age, 10/dose	2-butoxyethanol (CAS: 111-76-2) (purity unknown)	Different dosages(undiluted), single dermal application (occlusive), 24 h exposure, 14d post exposure observation period Mortality: Within 48h after application	560 mg/kg bw (480-640 mg/kg bw) Extreme congestion of the kidney, haemoglobinuria, pale liver, enlarged spleen	Mellon Institute of Industrial Research (1952) and Carpenter et al. (1956)
LD_{50}-Test, no guideline followed, but according to U.S. Federal Hazardous Substances Labelling Act (21 CFR 191)	**Rabbit,** (white) strain not given (1-4 kg), 4 animals/dose	2-butoxyethanol (CAS: 111-76-2) (purity commercial grade, 99.6 %)	3 dosages (undiluted) were tested, single dermal application (occlusive) to abraded skin	680 mg/kg bw (calculated by Finney, 1952)	Roudabush et al. (1965)
LD_{50}-Test, similar to OECD TG 402	**Rabbit,** New Zealand White, 10 animals	Polysolv EB (2-butoxyethanol, purity unknown)	Single dermal application (occlusive) of (1) 2000 mg/kg bw, 24h exposure, epidermal abrasions were made every 2-3 cm over the exposed area (sufficiently deep to penetrate the stratum corneum but not deep enough to produce bleeding), 14d post exposure observation period After 24h, all rabbits exhibited lacrimation, bloody urine, flaccid muscle tone and anorexia, mortality of all rabbits	(1) < 2000 mg/kg bw (2) 580 mg/kg bw	MB research laboratories (1976)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, purity**	**Dose levels duration of exposure**	**Value LD_{50}**	**Reference**
			during the second day of observation (2) the same conditions, but the doses tested were 250, 500 and 1000 mg/kg bw/d Mortality: 250 mg/kg bw: 0, 500 mg/kg bw: 1/4, 1000 mg/kg bw/d: 4/4, blood in urine, flaccid muscle tone and anorexia, necropsy: blood in urine, liver and renal injuries		
LD_{50}-Test, similar to OECD TG 402	**Rabbit,** New Zealand White, 6 animals	2-butoxyethanol (CAS: 111-76-2) (purity >99.5 %)	Single dermal application (undiluted) of 72, 90, 108, 135, 180 and 225 mg/kg bw, 8h exposure, 14d post exposure observation period Mortality: 72/90 mg/kg: 2/6, 108 mg/kg: 4/6, 135/180 mg/kg: 5/6, 225 mg/kg: 6/6; death occurred between day 1 and day 8, caused by renal impairment, Clinical signs: Prostration, hypothermia and haemoglobinuria Necropsy: Congestion of the liver, necrotic foci with mesenchymatous reactions and inconstant steatosis, passive congestion of the spleen, enlarged kidney with haemoglobinemic nephrosis, cutaneous lesions including necrosis	100 mg/kg bw (calculated)	Duprat and Gradiski (1979)
LD_{50}-Test, similar to OECD TG 402	**Rabbit,** New Zealand White, 4 males/group	2-butoxyethanol (CAS: 111-76-2) (purity unknown)	Single dermal application (occlusive) of 900 or 450 mg/kg bw, 24h exposure Mortality: 450 mg/kg bw: 1/4, 900 mg/kg bw: 4/4, Necropsy: Orange red lung and liver, dark spleen, dark red kidneys, orange peritonea and intestine, blood in urine	569 mg/kg bw	Bushy Run Research Center (1980b)
LD_{50}-Test, similar to OECD	**Rabbit,** New Zealand	2-butoxyethanol (CAS: 111-76-	Single dermal application (occlusive) of 153, 307,	435 mg/kg bw (calculated)	Eastman Kodak (1981b)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, purity**	**Dose levels duration of exposure**	**Value LD_{50}**	**Reference**
TG 402; nine glycol ethers were tested	White, 5/group	2) (purity >99.5 %)	614 and 1239 mg/kg bw, 24h exposure, 14d post exposure observation period Clinical signs: 153 mg/kg bw: Anorexia, depression, cyanosis and ataxia, ≥307 mg/kg bw: Salivation, nasal discharge, iritis, depression, laboured breathing and prostration, Necropsy: ≥614 mg/kg bw: Renal, hepatic and thymic effects, blood in urinary bladder		
LD_{50}-Test, similar to OECD TG 402, GLP study	**Rabbit,** New Zealand White, 5/sex/group	2-butoxyethanol (CAS: 111-76-2) (purity unknown)	Single dermal application (semi-occlusive) of 1000 or 2000 mg/kg bw, 24h exposure, 14d post exposure observation period Mortality: 1000 mg/kg bw: 0/10, 2000 mg/kg bw: 1/5 females, 1 male and 1 female were killed in extremis 2 days after dosing, Clinical findings: Lethargy, red stained urine, laboured breathing, hunched posture and isolated incidents of loss of righting reflexes, hypothermia, ataxia and diarrhoea Necropsy: 2000 mg/kg bw: Hepatic and renal toxicity, haemorrhage of the gastric mucosa, of the non-glandular epithelium of the stomach, of the small and large intestine, red fluid in the urinary bladder	> 2000 mg/kg bw	Safepharm laboratories (1994a)
LD_{50}-Test, similar to OECD TG 402, GLP study	**Rabbit,** New Zealand White, 5/sex/group	2-butoxyethanol (CAS: 111-76-2) (purity unknown)	Single dermal application (occlusive) of 500, 702 or 1000 mg/kg bw, 24h exposure, 14d post exposure observation period Mortality: 1000 mg/kg bw: 3 animals were killed in extremis,	841 mg/kg bw for males and females 1060 mg/kg bw for males 667 mg/kg bw for females	Safepharm laboratories (1994a)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, purity	Dose levels duration of exposure	Value LD_{50}	Reference
			Clinical signs: Ataxia, hunched posture, lethargy, laboured breathing, diuresis, red-coloured urine and skin and eyes pale yellow in appearance, Necropsy: Haemorrhagic lungs, dark or pale liver, dark kidneys, red liquid in the urinary bladder, Skin: Irritation, very slight to well defined erythema, very slight to severe oedema, scattered areas of grey/green-coloured dermal necrosis, desquamation, slight haemorrhage of dermal capillaries, small superficial scabs		

9.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

Animal studies

In rats, three studies showed LD_{50}-values greater than 2000 mg/kg bw. In the most recent studies, performed according to the same experimental protocol except for occlusion (one occlusive and the other semi-occlusive) animals exhibited clinical signs only when exposed to 2-butoxyethanol under complete occlusion. Clinical signs were haemolysis, lethargy, ataxia, and signs of hepatic and renal toxicity.

In guinea-pigs, LD_{50}-values ranged from 208 to 6411 mg/kg bw. Only one recent study was performed according to standard guidelines. This study gave a LD_{50} of greater than 2000 mg/kg bw. For this study, no adverse effects were described (local or systemic). Very few details are available about the local or systemic toxicity for the other studies.

In rabbits, except for one study (Duprat and Gradiski, 1979), which shows a very low LD_{50} of 100 mg/kg bw, results were quite consistent. When 2-butoxyethanol was applied occlusively, calculated LD_{50} for a 24-hour application ranged from 435 to 841 mg/kg bw in 6 studies. When applied semi-occlusively, LD_{50} was greater than 2000 mg/kg bw, showing the importance of evaporation. Common systemic signs of toxicity usually seen with 2-butoxyethanol were described: Ataxia, laboured breathing, depression, cyanosis, sign of toxicity in the kidney, liver, thymus and spleen. Local signs of irritation were seen in some studies, mild irritation for lower doses and sometimes severe irritation, even necrosis for the higher doses.

No human data on acute dermal toxicity is available.

Overall, for dermal toxicity, differences were seen between the tested species and the mode of occlusion. In synopsis of the available data the rabbit seems to be the most sensitive species compared

to the rat and guinea pig to the acute dermal toxic effects of 2-butoxyethanol. In rabbits LD_{50}-values of about 500 mg/kg bw were noted when administered occlusively whereas rats and guinea pigs exhibited LD_{50}-values greater than 2000 mg/kg bw in the same experimental conditions. Therefore LD_{50}-values for classification of 2-butoxyethanol were derived from acute dermal toxicity studies in the most sensitive species the rabbit.

9.2.2 Comparison with the CLP criteria

Acute dermal toxicity means those adverse effects occurring following dermal administration of a single dose of a substance or a mixture, or multiple doses given within 24 hours. Acute toxicity relates to effects occurring after a single or relative brief exposure to a substance or mixture. Acute toxicity classification is generally assigned on the basis of evident lethality. The evidence for acute dermal toxicity of 2-butoxyethanol is obtained from animal testing. Human data on acute dermal toxicity of 2-butoxyethanol is not available. Substances can be allocated to one of four toxicity categories based on acute toxicity by the dermal route according to the criteria shown in the Table 3.1.1 of Annex I, Part 3, Table 3.1.1 of CLP.

The following applies for the classification as

'Acute dermal toxicity - Category 3: 200 < ATE ≤ 1000 mg/kg bw'

'Acute dermal toxicity - Category 4: 1000 < ATE ≤ 2000 mg/kg bw.'

The lowest LD_{50}-values for classification of 2-butoxyethanol for the dermal route was derived from studies in rabbits, which ranged from 435 to 841 mg/kg bw after a 24-hour application. Based on the review of the available experimental data for acute dermal toxicity for 2-butoxyethanol, it is concluded that 2-butoxyethanol meets the criteria for classification as Acute Tox. 3, H311 according to CLP (Annex I, Part 3, Table 3.1.1 Acute toxicity Category 3 (dermal): 200 < ATE ≤ 1000 mg/kg bw).

9.2.3 Conclusion on classification and labelling for acute dermal toxicity

According to CLP 2-butoxyethanol has to be classified as:

Acute Tox. 3 for dermal exposure and labelled with hazard statement H311: Toxic in contact with skin.; with the pictogram "GHS06: Skull and crossbones", and with the signal word "Danger".

Acute toxicity Estimate (ATE, dermal)

According to Annex I, Part 3, Point 3.1.3.6. ('Classification of mixtures based on ingredients of the mixture (Additivity formula)') classification of mixtures is based on the calculated ATE of a mixture. The ATE for a mixture is determined by calculation from the ATE values when data available for all relevant ingredients or when data are not available for all components according to the appropriate additivity formula (s. CLP Annex I, Part 3, and Point 3.1.3.6.1. or 3.1.3.6.2.3).

For the classification for acute dermal toxicity of mixtures containing 2-butoxyethanol an ATE value of 300 mg/kg bw is proposed for the calculation with the additivity formula according to Annex I, Part 3, and Point 3.1.3.6.1. or 3.1.3.6.2.3 of the CLP. The ATE value (dermal) for 2-butoxyethanol is converted from the acute toxicity point estimate of acute hazard category 3 (see Table 3.1.2 in the CLP Regulation).

Justification for the converted ATE value (dermal) of 300 mg/kg bw:

There are significant differences in the acute dermal toxicity of 2-butoxyethanol to different species. Data on three species is available: Rat, rabbit and guinea pig. In rats, the available data unequivocally show an LD_{50} greater than 2000 mg/kg bw under all exposure conditions. In guinea pigs, variations were seen depending on the studies. The LD_{50} ranged from 230 mg/kg bw to higher than 2000 mg/kg bw. In rabbits, results were generally consistent. When 2-butoxyethanol was applied occlusively, the calculated LD_{50} for a 24-hour application ranged from 435 to 841 mg/kg bw. Human data on acute dermal toxicity is not available. When 2-butoxyethanol was administered non-occlusively or semi-occlusively the LD_{50} was much higher than when administered occlusively. As for acute oral toxicity, the rabbit seems to be a particularly sensitive species when administered occlusively, whereas the other species (rat and guinea pig) for which data is available exhibited LD_{50} values generally greater than 2000 mg/kg bw under the same experimental conditions.

In this case conversion from the experimentally obtained acute toxicity range value (or acute toxicity hazard category) to an acute toxicity point estimate for use in the formulas (CLP Annex I, Part 3, and Point 3.1.3.6.1. or 3.1.3.6.2.3) for the classification of mixtures is applied. According to CLP, Annex I, Table 3.1.2. the converted acute toxicity point estimate for dermal administration classified in the hazard Category 3 is 300 mg/kg bw (see Note 1: 'These values are designed to be used in the calculation of a mixture based on its components and do not represent test results.').

Accordingly, the converted ATE (dermal) value of 300 mg/kg bw should be used in the respective additivity formula for the classification of mixtures containing 2-butoxyethanol.

9.3 Acute toxicity - inhalation route

Table 13: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, , form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value** **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 more data	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:	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**	**Value** **LC_{50}**	**Reference**
			3/6 during 14d post exposure period 202 ppm, m+f: 0/6 Necropsy: died animals: enlarged and discoloured kidneys, urinary bladder filled with red stained urine		
LC_{50}-Test, 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 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 %, satured 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 Blood: Hb concentration 35 to 50 % of the normal Mortality: m/f: 4/4 on Day 2	LC_{100} = 2400 ppm, 5h (corresponding to 2585 ppm, 4h = 12.62 mg/L/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**	**Value** **LC_{50}**	**Reference**
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 protocol missing from publication.	**Mouse**, strain and number of animal used not given	2-butoxyethanol (CAS: 111-76-2), purity commercial grade, vapour	Clinical signs: Dyspnoea, haemglobulinurea, death were noted in the 4th wk after exposure Necropsy: Findings in spleen, liver, lungs, and kidneys Wistar rats (23 animals/group, gender not specified) were exposed to 0, 135, or 320 ppm Exposure duration: 7 hours/day, 5 days/week for 5 weeks (Werner et al., 1943, 597282). Haematologic endpoints—RBC, WBC, differential, and reticulocyte counts and Hb concentration—were evaluated. Exposure to 320 ppm 2-butoxyethanol resulted in an increased percentage of circulating immature granulocytes, decreased Hb concentrations and RBC counts, and increased reticulocyte counts. These haematologic changes were not severe; they were reversed 3 weeks after discontinuing exposure. No effect on WBC count was observed. In another study, Werner et al. (1943, 100219) exposed groups of two dogs of	700 ppm, 7h (corresponding to 843 ppm/4h = 4.12 mg/L/4h)	Werner et al. (1943) cited in Carpenter et al. (1956)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, , form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value** LC_{50}	**Reference**
			unspecified strain to subchronic inhalation doses of 0 or 415 ppm 2-butoxyethanol 7 hours/day, 5 days/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.		
LC_{50}-Test, no guideline followed	**Guinea pig,** strain unspecified, adult	2-butoxyethanol (CAS: 111-76-2), "Substantially saturated vapour"	1300 ppm for 7h, whole body exposure, 14d post exposure period	1300 ppm, 7h (corresponding to 1566 ppm/4h = 7.65 mg/L/4h)	Mellon Institute of Industrial Research (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)	Gingell et al. (1998)

Table 14: Summary table of human data on acute inhalation toxicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	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 8h	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 data	2-butoxyethanol (CAS: 111-76-2), purity commercial grade, vapour	Exposure of 4 male volunteers to 50 ppm (0.24 mg/L) for 2h in an open-system exposure chamber	50 ppm: No consistent effects on the lungs (ventilation or breathing rate) or the heart (electrocardiogram readings or heart rate)	Johanson (1986)
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 vapour inhalation (through the mouth alone)	50 ppm: No overt signs of toxicity	Johanson and Boman (1991)

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

Animal studies

A number of studies are available to assess the LC_{50} of 2-butoxyethanol, although not all of these are for the preferred exposure time of 4 hours. For direct comparison with the classification criteria, LC50 values need to be adjusted to a 4-hour equivalent using Haber's law ($C^n*t=k$). The value of n, which is specific to individual substances, should be chosen using expert judgement. If an appropriate value of n is not available in the literature, the Guidance on IR/CSA, Section R.7.4.4.1 recommends to set n = 3 for extrapolation to shorter duration and to set n = 1 for extrapolation to longer duration. Good information is available for rats and guinea pigs. Data is also available for mice.

For rats, four reliable studies are available. In rats, the lowest LC_{50} of 2-butoxyethanol was calculated between 450 ppm (= 2.2 mg/L/4h) in females and 486 ppm (2.37 mg/L/4h) in males for a 4 hour exposure (Bushy Run Research Center, 1980a). Other studies give results quite consistent with this one. In these studies, females and old animals (2.21 mg/L/4h) were more sensitive than males or young animals (4.92 mg/L/4h). Clinical symptoms and pathology were: Lethargy, ataxia, laboured breathing, and loss of coordination, haemolysis and tail necrosis. Renal injuries were commonly seen during pathological examinations.

A GLP study is available in guinea pigs and this showed no deaths for exposures at the maximum practical vapour concentration achievable 633 ± 14.2 ppm in males and 691 ± 37.6 ppm in females (around 3.2 mg/L) but the exposure time was only one hour. In another study with guinea pigs the LC_{50} value after a 7 hour exposure was observed at 1300 ppm, extrapolated to 4 hours using the Haber equation, indicates the LC_{50} of 1566.59 ppm (= 7.65 mg/L/4h).

In the single study available in mice, the results from the 7 hour exposure, if extrapolated to 4 hours using the Haber equation, indicate the LC_{50} of 843.55 ppm (= 4.12 mg/L/4h).

Human data

Acute human toxicity data were reported from volunteers for determination of pharmacokinetic data. The symptoms reported by the volunteers were signs of irritation (throat and ocular) and headache. These symptoms did not seem to be dose-related. No overt signs of systemic toxicity were noted after exposure of two 4 hours to 195 ppm (0.95 mg/L).

9.3.2 Comparison with the CLP criteria

Acute inhalation toxicity means those adverse effects occurring following an exposure by inhalation over 4 hours to a single concentration of a substance or a mixture. Acute toxicity relates to effects occurring after a single or relative brief exposure to a substance or mixture. Acute toxicity classification is generally assigned on the basis of evident lethality. The evidence for acute inhalation toxicity of 2-butoxyethanol is obtained from animal testing. Human data on acute inhalation toxicity of 2-butoxyethanol relevant for classification is not available. Substances can be allocated to one of four toxicity categories based on acute toxicity by inhalation according to the criteria shown in the Table 3.1.1 of Annex I, Part 3, Table 3.1.1 of CLP.

The following applies for the classification as

'Acute inhalation toxicity - Category 3 (vapour): $2.0 < ATE \leq 10.0$ mg/L'

'Acute inhalation toxicity - Category 4 (vapour): $10.0 < ATE \leq 20.0$ mg/L.'

The rat is considered being the most sensitive species to acute inhalation toxicity of 2-butoxyethanol among the other species tested. In the rat the lowest LC_{50} values are 450 ppm (= 2.2 mg/L/4h) in females and 486 ppm (2.37 mg/L/4h) in males for a 4 hour exposure. Based on these lowest LC_{50} values from the studies with rats, 2-butoxyethanol fulfils the criteria for classification for acute inhalation toxicity Category 3. The studies with guinea pigs and mice give results quite consistent with these in rats.

Therefore, it is concluded that 2-butoxyethanol meets the criteria for classification as Acute Tox. 3, H331 according to CLP (Annex I, Part 3, Table 3.1.1 Acute toxicity Category 3 (inhalation): $2.0 < ATE \leq 10.0$ mg/L).

9.3.3 Conclusion on classification and labelling for acute inhalation toxicity

According to CLP 2-butoxyethanol has to be classified as:

Acute Tox. 3 for exposure by inhalation and labelled with hazard statement H331: Toxic if inhaled.; with the pictogram "GHS06: Skull and crossbones", and with the signal word "Danger".

Acute toxicity Estimate (ATE, inhalation)

According to Annex I, Part 3, Point 3.1.3.6. ('Classification of mixtures based on ingredients of the mixture (Additivity formula)') classification of mixtures is based on the calculated ATE of a mixture. The ATE for a mixture is determined by calculation from the ATE values when data available for all relevant ingredients or when data are not available for all components according to the appropriate additivity formula (s. CLP Annex I, Part 3, and Point 3.1.3.6.1. or 3.1.3.6.2.3).

For the classification for acute inhalation toxicity of mixtures containing 2-butoxyethanol an ATE value (vapours) of 3 mg/L/4h is proposed for the calculation with the additivity formula according to Annex I, Part 3, and Point 3.1.3.6.1. or 3.1.3.6.2.3 of the CLP. The ATE value (inhalation, vapours) for 2-butoxyethanol is converted from the acute toxicity point estimate of acute hazard category 3 (see Table 3.1.2 in the CLP Regulation).

Justification for the converted ATE value (dermal) of 3 mg/L/4h:

LC_{50} values of 2-butoxyethanol were derived from data on three species: Rat, mouse and guinea pig. The majority of the available studies did not use the defined exposure time of 4 hours to assess the LC_{50}. The lowest LC_{50} values were observed in rats. For rats, the 4-hour LC_{50} is calculated at 450 ppm (= 2.2 mg/L/4h) in females and 486 ppm (2.37 mg/L/4h) in males. In guinea pigs, no deaths are seen after exposures to about 633 ppm in males and 691 ppm in females (around 3.2 mg/L) over an exposure time of only one hour. In another study with guinea pigs the LC_{50} value after a 7 hour exposure was observed at 1300 ppm (extrapolated to 4 hours using the Haber equation: 1566.59 ppm (= 7.65 mg/L/4h). In mice, the results from the 7 hour exposure, if extrapolated to 4 hours indicate the LC_{50} of 843.55 ppm (= 4.12 mg/L/4h). The results of the available and accepted studies in the three species showed LC_{50} values over a range of 2.2 to 7.65 mg/L/4h. Based on these data, 2-butoxyethanol fulfils the criteria for classification for acute inhalation toxicity Category 3. It was concluded that the studies with guinea pigs and mice give results quite consistent with these in rats.

In this case conversion from the experimentally obtained acute toxicity range value (or acute toxicity hazard category) to an acute toxicity point estimate for use in the formulas (CLP Annex I, Part 3, and Point 3.1.3.6.1. or 3.1.3.6.2.3) for the classification of mixtures is applied. According to CLP, Annex I, Table 3.1.2. the converted acute toxicity point estimate for exposures by inhalation classified in the hazard Category 3 is 3 mg/L/4h (see Note 1: 'These values are designed to be used in the calculation of a mixture based on its components and do not represent test results.').

Accordingly, the converted ATE (inhalation) value of 3 mg/L/4h should be used in the respective additivity formula for the classification of mixtures containing 2-butoxyethanol.

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).

9.4 Skin corrosion/irritation

Table 15: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
OECD TG 404 (Acute Dermal Irritation/Corrosion) No GLP compliance (study considered reliable with restrictions) Deviations from guideline: Fur removed 7 days before treatment (OECD TG 404: 24 h); treatment: occlusive coverage with teflon exposure chamber fixed with tape for the duration of the exposure period instead of semi-occlusive patch dressing (Section 12, OECD TG 404); no information if controls were also washed with detergent solution and water.	**Rabbit,** New Zealand White, Sex not specified, 5 animals	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	0.5 ml non-diluted test substance/skin area (6 cm^2) using Teflon exposure chambers (occlusive); other side of spinal column served as control. Washing of test areas with water and detergent after 4h of treatment. Observations: 1, 24, 48 and 72 h after treatment and twice a week until termination of study (14 days after exposure).	Erythema score (mean of 24/48/72h and all 5 animals): 1.7 (max. score: 4.0), not fully reversible within 14 days of observation. Oedema sore (mean of 24/48/72h and all 5 animals): 0.13 (max. score: 4.0), not fully reversible within 48 h of observation. Maximum degree of eschar formation (mean of 24/48/72h and all 5 animals): 2.0; no data on reversibility of effects. No individual scores reported, but it was observed that results per animal were very divergent, from not irritating to very irritating. No effects in controls.	Jacobs et al. (1987) and Jacobs and Martens (1987)
CFR title 16, section 1500.41 (Method of testing primary irritant substances) No GLP compliance (study considered reliable with restrictions) Deviations from guideline: No - Erythema/oedema scores and gradings identical compared to OECD TG 404	**Rabbit,** albino (not specified), Sex not specified, 6 animals	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	0.5 ml non-diluted test substance/skin area (per animal both, abraded and intact skin areas were treated) - Abrasions: minor incisions to stratum corneum but not sufficiently deep to cause bleeding Occlusive coverage Exposure duration: 24 h Observations: 24 h and 72 h after	Primary dermal irritation index (PDII) according to Draize protocol (mean of 24 and 72h and all 6 animals): 1.57 (max. score: 8.0) Erythema scores (max. score: 4.0): 24 h: -intact skin: 1.0; - abraded skin: 1.0; 5/6 animals exhibited slight to moderate erythema (abraded and intact skin) 72 h: -intact skin: 0.83; - abraded skin: 0.83; 4/6 animals exhibited very slight to moderate erythema (abraded and intact skin); -not fully reversible within 72	Grote (1979a)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
			test start No controls	h. Oedema scores (max. score: 4.0): 24 h: -intact skin: 0.67; - abraded skin: 0.67; 4/6 animals exhibited slight oedema (abraded and intact skin) 72 h: -intact skin: 0.5; - abraded skin: 0.5; 3/6 animals exhibited slight oedema (abraded and intact skin); -not fully reversible within 72 h.	
In vivo test; no validated guideline followed (internal BASF test method) No GLP compliance (study considered reliable with restrictions) No definition of erythema gradings/scores reported	**Rabbit**, Vienna White, Sex not specified, 2 animals	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	non-diluted test substance on shaved skin area (2.5 cm^2; back) Occlusive coverage Exposure duration: 20 h Observations: immediately after exposure, and 1, 3, 8, and 14 days after exposure Controls: pure alcohol	Erythema score (mean of 2 animals and time points 24 h – 72 h): 2.0 (max. 4.0). Effects were persistent and not reversible within 14 days. No results of control treatment reported. No individual scores reported.	BASF AG (1960)
Main test (part A): EU Method B.4 (Acute Toxicity: Dermal Irritation/Corrosion), Additional test (part B): Draize test for skin irritancy; not performed according to any current validated OECD TG 4 No GLP compliance	**Rabbit,** New Zealand White, Sex not specified, 3 animals for EU method B.4 6 animals for Draize test	2-butoxyethanol, (CAS: 111-76-2), (purity: 99%)	0.5 ml non-diluted test substance/shaved skin area (other side of spinal column served as control) Occlusive coverage Exposure duration: - EU method B.4: 4 h	Main test (EU B.4): No individual or mean erythema/oedema scores reported for any observation time point. 2-butoxyethanol **classified as irritant** based on the following criteria: - Erythema or eschar formation, or - Oedema equivalent to a mean value of 2 or more observed in 2 or more animals (mean of all observation time points).	Zissu (1995)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
(study considered reliable with restrictions) Deviations from guidelines: EU method B.4: no Draize protocol: not specified			- Draize test: 24 h Observations: - EU method B.4: 24, 48 and 72 h after application - Draize test: 24 and 72 h after application	Draize test: **Primary dermal irritation index** (PDII) according to Draize protocol for skin irritation (mean of 24 and 72h and all 6 animals for both abraded and intact skin): **7.5** (max. score: 8.0)	
In vivo test; no validated guideline followed. No GLP compliance (study considered not reliable) Grading of irritation not according to any known (current or former) system (1 - 10)	**Rabbit**, Sex and strain not specified 5 animals	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	0.01 mL applied openly on clipped area of the rabbit belly. Concentration not reported Exposure duration not reported Observation: 24 h after exposure	No irritation in 3 rabbits 2 rabbits showed moderate capillary injection Insufficient data for quantitative interpretation and classification Method and reporting not adequate enough to evaluate skin irritation	Bushy Run Research Center (1989)
In vivo test; method not specified; no validated guideline followed. GLP compliance not specified (study not assignable)	**Rabbit** New Zealand White, Sex: female No. of animals not specified	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	Exposure method not specified; Concentration: ≥ 72 mg/kg Exposure duration: 8 h Observations: not specified, day 4 and 14 after exposure.	- Development of cutaneous lesions on day 4 - Necrosis of epidermis and dermis on day 4 - Skin lesions healed within 14 days	Duprat and Gradiski (1979)
EU Method B.4 (Acute Toxicity: Dermal Irritation/Corrosion) GLP compliance not specified (study not assignable)	**Rabbit** New Zealand White, Sex: male 6 animals	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	0.5 mL undiluted test substance/intact skin Exposure duration: 4 h Observations: 5 h, 1, 3 and 7 d after exposure	Variable results - Severe and persistent erythema with eschar and severe oedema in 3 rabbits - Slight oedema and erythema in 3 rabbits - No oedema after 7 days of observation Insufficient data for quantitative interpretation and classification	Rohm and Haas Co. (1989)
In vivo test;	**Rabbit**	2-butoxyethanol,	0.3 g/ kg bw test material/ clipped	Moderately irritating	Eastman Kodak

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
method not specified GLP compliance not specified (study not assignable)	Sex and strain not specified No. of animals not specified	(CAS: 111-76-2), (purity unknown)	skin Occlusive patch Exposure duration: 24 h Observation time points not specified	Insufficient data for quantitative interpretation and classification Method and reporting not adequate enough to evaluate skin irritation	(1981b)
In vivo test; method not specified; no validated guideline followed. No GLP compliance (study not assignable)	**Guinea pig** Sex and strain not specified No. of animals not specified	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	1, 5, 10 and 20 ml/kg bw on depilated skin Occlusive patch Exposure duration: 24 h Observation time points not specified	Strongly irritating Insufficient data for quantitative interpretation and classification Method and reporting not adequate enough to evaluate skin irritation	Eastman Kodak (1981b)
In vivo test; method not specified; no validated guideline followed. GLP compliance not specified (study not assignable)	**Guinea pig** Sex and strain not specified No. of animals not specified	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	10% and 20% test substance in 0.9% saline/skin area Occlusive patch Exposure duration not specified Observation time points not specified	- 25% solution irritating - 10% solution non-irritating Insufficient data for quantitative interpretation and classification Method and reporting not adequate enough to evaluate skin irritation	Unilever Research (1989)

Table 16: Summary table of human data on skin corrosion/irritation

Type of data/report	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Human repeated patch test for evaluating sensitising effects; publication	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	Tests substance was tested at 10 % aq. v/v solution (10%: at that time highest concentration used in cosmetic products) 214 adults 13 adults were discontinued for reasons unrelated to treatment Induction phase and first reading: 0.2 mL test solution, occlusive patch on the	After 24 h: - No reaction in 199 of 203 adults - Slight erythema in 3 of 203 adults - Definite erythema in 1 of 203 adults	Greenspan et al. (1995)

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		back Exposure duration: 24 h Observation: 24 h after exposure		
Test of percutaneous absorption of 2-butoxyethanol in humans; publication	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	Subjects: 5 healthy men; Exposure: fingers of left hand were placed in pure test substance (in a polyethylene jar through cut holes in a polyethylene cap). Fingers of right hand served as control. Exposure duration: 2 h (at 21 °C); Washing of hands using water and mild soap after exposure period; Observations: at regular intervals, not specified; Measured parameters: - skinfold thickness of dorsal skin on third phalanx - finger volume using plethysmograph	No irritation of skin, but skin appeared: - more rigid - less elastic - more wrinkled Effects reached maximum 2 - 4 h after exposure and gradually disappeared. Finger volume decreased significantly after exposure (max. 2 h after exposure), but returned to normal 1 day later. A dry, reticulate pattern with small fissures developed within a few hours after exposure; in some cases fissures became slightly erythematous. Effects disappeared within 1 – 2 d. One subject developed white fingers during exposure.	Johanson et al. (1988)
Test of percutaneous absorption from aqueous solutions of 2-butoxyethanol in humans; publication	2-butoxyethanol, (CAS: 111-76-2), (purity: 99 %)	Occlusive application of 8 ml of 50 %, 90 % or 100% 2-butoxyethanol solution using bottomless glass chambers (40 cm^2) Chambers were glued to the skin. Exposure duration: 4 h. Observation time points: not specified	- In none of the volunteers skin irritation occurred - After exposure the skin had a wrinkled appearance.	Jakasa et al. (2004)

Table 17: Summary table of other studies relevant for skin corrosion/irritation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
LD50-Test according to OECD TG 402, GLP	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	Single dermal application (occlusive and semi-occlusive) Concentration: 2g/kg bw **Rat,** Sprague-Dawley,	No signs of skin irritation Insufficient data for quantitative interpretation and classification	Safepharm laboratories (1993a) and Safepharm

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
compliant; Study report		5animals/sex Exposure duration: 24 h Observation period: 14 d post exposure Observations: 0.5, 1, 2, 4 h after exposure, then daily until the end of the study.		laboratories (1993b)
LD50-Test according to OECD TG 402, GLP compliant; Study report	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	Single dermal application (occlusive) Concentration: 500, 707 or 1000 mg/kg bw and Single dermal application (semi-occlusive) Concentration: 1 and 2 g/kg **Rabbit**, New Zealand White, 5 animals/sex/group Exposure duration: 24 h Observation period: 14 d post exposure Observations: 0.5, 1, 2, 4 h after exposure, then daily until the end of the study.	Semi-occlusive treatment (2 g/kg) and occlusive treatment (concentration not specified): - Very slight to well defined erythema at dose site - slight to severe oedema at dose site - scattered areas of black or green necrosis - slight haemorrhage of the dermal capillaries - hardened scabs over dried blood and desquamation - light brown discoloration of the epidermis or small areas of light brown discoloration and crust formation Insufficient data for quantitative interpretation and classification	Safepharm laboratories (1994a) and Safepharm laboratories (1994b)
LD50-Test according to OECD TG 402, No GLP compliance; Study report	2-butoxyethanol, (CAS: 111-76-2); (purity unknown)	Single dermal application (occlusive) Concentration: 0.5 and 1.0 mL/kg bw **Rabbit,** New Zealand White, Sex: male 4 animals Exposure duration: not specified Observations: not specified	High dose: erythema and necrosis at application site Insufficient data for quantitative interpretation and classification	Bushy Run Research Center (1989)

9.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

Animal studies

There is one *in vivo* skin irritation/corrosion study for 2-butoxyethanol in rabbits available, which is performed according to OECD TG 404 (version 1981), not compliant with GLP (Jacobs and Martens,

1985; Jacobs et al., 1987). Thus, the study is considered relevant and reliable with restrictions. 2-butoxyethanol caused an erythema score of 1.7 (mean of 24 h to 72 h and all 5 animals), an oedema score of 0.13 (mean of 24 h to 72 h and 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 are not reported, but considering the mean eschar score of 2.0, respectively, it can be derived, that in at least 2/5 animals the eschar score was > 2.0. Moreover, results per animal were reported to be very divergent, from not irritating to very irritating. Effects were, furthermore, persistent and not fully reversed at the end of the 14-day observation period. No effects were noted in control animals. The results of the study indicate that 2-butoxyethanol is a mild to moderate skin irritant and exposure to this substance results in slight to moderate erythema, but only very slight oedema. However, information is missing on the exact control treatment (e.g. washing after treatment). Furthermore, there are some additional deviations to the OECD TG 404 (Table 15), including the early fur removal and the occlusive coverage during treatment using teflon exposure chambers. An occlusive patching for the application of the test substance, which was part of the OECD standard protocol before 1987, results in more rigorous test conditions compared to the semi-occlusive patching used today. Thus, the method of application should be accounted for in the evaluation of effects. Because the irritating effects observed in this study were not severe despite the occlusive patching, those deviations to the guideline are considered to not interfere with the reliability of the study.

Another study in rabbits is available (Grote, 1979a), exposing animals occlusively to 2-butoxyethanol for a longer period than the 4 h exposure period recommended in current, validated OECD TG 404 (24 h). The study was performed according to a protocol (CFR title 16, section 1500.41) of the USA Federal Hazardous Substances Act (US-FHSA), not according to GLP. Although this test involves a 24-hour test material exposure followed by observations at 24 h and at 72 h at the termination of the experiment and does not include a 48-hour observation time, it is feasible to use this data for classification. For this purpose, mean values for erythema and oedema are calculated on the basis of only the two time points and information on the reversibility of effects is evaluated. Since in this test the test material is patched both on abraded and on intact skin of rabbits, calculation of mean scores are restricted to the results obtained from intact skin. Grote (1979a) calculated a mean erythema score of 1.0 after 24 h and 0.83 after 72 h for the intact skin area. In total, 5/6 animals exhibited a slight to moderate erythema at 24 h, whereas at 72 h 4/6 animals exhibited a very slight to moderate erythema. Moreover, an oedema score of 0.67 was obtained for the intact skin after 24 h (4/6 animals) and the oedema score after 72 h was 0.5 (3/6 animals). Effects were not reversible within the 72 h observation period.

Zissu (1995) similarly demonstrated an irritating potency for 2-butoxyethanol in rabbits using OECD TG 405. However, no individual or mean erythema/oedema scores were reported for any observation time point. Classification was rather based on the following criteria: either erythema or eschar formation, or oedema equivalent to a mean value of 2 or more observed in 2 or more animals. Due to this missing information and also due to the missing statement about the reversibility of effects, results of this study cannot be used for a conclusive classification of 2-butoxyethanol as irritating/corrosive to the skin. However, because the above mentioned appropriate and reliable tests yielded in similar results, the outcomes of this study can be taken into account as supportive data.

Furthermore, two other studies in rabbits are available (BASF AG, 1960; Zissu, 1995), exposing animals occlusively to 2-butoxyethanol for a longer period than the 4 h exposure period recommended in current, validated OECD TG 404 (e.g. 20 -24 h). All results indicate that 2-butoxyethanol is slightly irritating to the skin, however due to the missing individual erythema/oedema scores, the prolonged exposure periods (e.g. 20 – 24 h versus 4 h), and in some cases the shortened observation period (e.g. 72 h versus 14 d) in comparison to the current and validated OECD TG 404, a classification according to CLP Regulation (Table 3.2.2) is inconclusive

using these study results. Nevertheless, because the above mentioned appropriate and reliable "Acute Dermal Irritation/Corrosion test" (OECD TG 404) and "Method of testing primary irritant substances (CFR title 16, section 1500.41) resulted in similar outcomes, the outcomes of these additional studies can be taken into account as supportive data.

A number of further *in vivo* skin irritation/corrosion studies are available for 2-butoxyethanol in rabbits and guinea pigs, but many of these studies are not assignable regarding their reliability (see Table 15) and, thus, do not provide sufficient information for quantitative interpretation and 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

3 studies are available evaluating the skin irritating effects of 2-butoxyethanol in humans directly by employing the human (repeated) patch test (Greenspan et al., 1995) or indirectly as side effect in absorption and toxicokinetic studies (Jakasa et al., 2004; Johanson et al., 1988). In the studies regarding substance absorption, no skin irritating effects of 2-butoxyethanol were detected, but skin appeared more wrinkled and less elastic after occlusive and immersive exposure, respectively. After immersion (Johanson et al., 1988), the finger volume 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).

9.4.2 Comparison with the CLP criteria

According to the CLP Regulation (Section 3.2.1.1), skin corrosion means the production of irreversible damage to the skin, following the application of a test substance for up to 4 hours. Skin irritation means the production of reversible damage to the skin following the application of a test substance for up to 4 hours.

On the basis of the results of animal testing a substance is classified as corrosive (Category 1), as shown in Table 3.2.1 of the CLP Regulation, if it produces destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, in at least 1 tested animal after exposure up to a 4 hour duration. Three subcategories are provided within the corrosive category: subcategory 1A, where responses are noted following up to 3 minutes exposure and up to 1 hour observation; subcategory 1B, where responses are described following exposure between 3 minutes and 1 hour and observations up to 14 days; and subcategory 1C, where responses occur after exposures between 1 hour and 4 hours and observations up to 14 days (Section 3.2.2.6.2., CLP Regulation).

On the basis of the results of animal testing a substance is classified as skin irritant (Category 2) (Table 3.2.2, CLP Regulation), if

- at least 2 of 3 (3 of 5, and 4 of 6, respectively) tested animals have a mean score of ≥ 2.3 - ≤ 4.0 for erythema/eschar or for oedema from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or

- inflammation persists to the end of the observation period normally 14 days in at least 2/3 (3/5, and 4/6, respectively) animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or

- there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

A separate irritant criterion accommodates cases when there is a significant irritant response but less than the mean score criterion for a positive test (e.g. at least 1 of 3 tested animals shows a very elevated mean score throughout the study, including lesions persisting at the end of an observation period of normally 14 days). Other responses could also fulfil this criterion. However, it should be ascertained that the responses are the result of chemical exposure (Section 3.2.2.8.1, CLP-Regulation). Moreover, when inflammation persists to the end of the observation period in 2 or more test animals, then a material shall be considered to be an irritant (Section 3.2.2.8.2, CLP-Regulation).

In the reliable *in vivo* assays performed according to OECD TG 404 (version 1981) (Jacobs and Martens, 1985; Jacobs et al., 1987) and CFR title 16, section 1500.41 (Grote, 1979a), respectively, mean erythema/oedema scores obtained for 2-butoxyethanol were below 2.3 and thus, according to the CLP Regulation (Annex I, Table 3.2.2) the substance shall be classified as not irritating to the skin based on this parameter. However, in both studies, as well as the supportive data, effects were persistent and not fully reversible by the end of the observation period (14 days 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, it can be concluded that the criteria for skin irritation Category 2 given in Table 3.2.2 in Annex I of the CLP Regulation are fulfilled for 2-butoxyethanol.

Although 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) supports the classification based on animal studies (Skin Irrit., Cat. 2).

Hence, for 2-butoxyethanol the classification as Skin Irrit. Category 2 H315 (Causes skin irritation) is justified.

9.4.3 Conclusion on classification and labelling for skin corrosion/irritation

According to CLP 2-butoxyethanol has to be classified as:

Skin Irrit. (Category 2) and labelled with hazard statement H315: Causes skin irritation.; with the pictogram “GHS07: Exclamation mark”, and with the signal word “Warning”.

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).**

9.5 Serious eye damage/eye irritation

Table 18: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
OECD TG 405, GLP compliant (study considered reliable without restrictions) Deviations from guideline: no	**Rabbit**, New Zealand White, Sex: female 3 animals	2-butoxyethanol, (CAS: 111-76-2), (purity: 99.6 %)	0.1 mL non-diluted test substance/eye (left eye served as control), Exposure duration: 24 h Washing with tap water after exposure, immediately before first reading. Observation period: 1, 24, 48, 72 h after treatment and after 7, 14 and 21 days	Chemosis score (mean of 24/48/72h) of the 3 test animals (max. score: 4.0): 2.0, 2.0, 1.3 Effects fully reversible within 14 days. Conjunctivae sore (mean of 24/48/72h) of the 3 test animals (max. score: 3.0): 2.3, 3.0, 2.3 Effects fully reversible within 21 days. Iris score (mean of 24/48/72h) of the 3 test animals (max. score: 2.0): 0.33, 1.0, 0.33 Effects fully reversible within 7 days. Cornea opacity score (mean of 24/48/72h) of the 3 test animals (max. score: 4.0): 1.0, 0.67, 1.0	BASF (2000)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
				Effects fully reversible within 21 days. Other effects: - blood discharge in 2 animals at time points 24, 48 and 72 h, disappeared by day 7. - Suppuration in 2 animals at time points 8 and 72 h, disappeared by day 7. No data on negative controls.	
CFR title 16, section 1500.42 (Test for eye irritants) No GLP compliance (study considered reliable with restrictions) Deviations from guideline: No Scoring system according to Draize test	**Rabbit**, Albino (strain not specified) Sex: not specified 6 animals	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	0.1 mL non-diluted test substance/eye (other eye served as control) Single instillation No washing of eyes Observations: 24, 48, 72 h and 7 d after instillation	Chemosis score (mean of 24/48/72h) of the 6 test animals (max. score: 4.0): 2.7, 3.0, 2.3, 2.7, 3.0, 2.7 Effects not fully reversible within 7 days. Conjunctivae sore (mean of 24/48/72h) of the 6 test animals (max. score: 3.0): 2.7, 2.7, 2.7, 2.0, 2.3, 2.3 Effects not fully reversible within 7 days. Iris score (mean of 24/48/72h) of the 6 test animals (max. score: 2.0): 0.7, 0.3, 0.0, 0.7, 0.7, 0.3 Effects not fully reversible within 7 days. Cornea opacity score (mean of 24/48/72h) of the 6 test animals (max. score: 4.0): 1.0, 1.7, N/A, 1.0, 1.0, N/A (N/A: Dulling of cornea seen in two animals at certain time points which prevented readings). Effects not fully reversible within 7 days. No data on negative controls.	Grote (1979b)
OECD TG 405 (version 1981), GLP compliance not specified (study considered reliable with restrictions) Deviations	**Rabbit**, New Zealand White, Sex: male and female 2x3 animals	2-butoxyethanol, (CAS: 111-76-2), (purity: 99 %)	2 tests with 3 animals each: 0.1 mL non-diluted test substance/eye (other eye served as control), No washing of eyes Observation period: 1, 24, 48,	Test 1: Chemosis score (mean of 24/48/72h and all 3 animals): 0.85 (max. score: 4.0), no data on reversibility Conjunctivae sore (mean of 24/48/72h and all 3 animals): 2.54 (max. score: 3.0), no data on reversibility Iris score (mean of 24/48/72h and all 3 animals): 1.0 (max. score: 2.0), no data on reversibility Cornea opacity score (mean of	Jacobs and Martens (1985) and Jacobs and Martens (1987) and Jacobs et al. (1989)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
from guideline: 2 tests with 3 animals; observations for only 7 days; no individual data reported			72 h after treatment and after 7 days	24/48/72h and all 3 animals): 1.59 (max. score: 4.0), no data on reversibility No individual scores reported. Test 2: Chemosis score (mean of 24/48/72h and all 3 animals): 0.89 (max. score: 4.0), no data on reversibility Conjunctivae sore (mean of 24/48/72h and all 3 animals): 2.51 (max. score: 3.0), no data on reversibility Iris score (mean of 24/48/72h and all 3 animals): 1.73 (max. score: 2.0), no data on reversibility No cornea opacity score given No individual scores reported. No data on negative controls. Other effects: - marked pannus in all 6 rabbits, not reversible (but not relevant for humans) - mean corneal upper layer damage was 95% after 4 h (average of 69% over the 24, 48 and 72 hour observations)	
OECD TG 405 (version 1981), GLP compliance not specified (study considered reliable with restrictions) Deviations from guideline: Observation for only 7 days, only 4 days reported; no individual data reported	**Rabbit**, New Zealand White, Sex: not specified No. of animals not specified, thought to be 6	2-butoxyethanol, (CAS: 111-76-2), (purity: 99 %)	0.1 mL non-diluted test substance/eye (other eye served as control), Exposure duration/Washing: not specified, but not assumed Observations: 4 24, 48, 72, and 96 h after instillation	Chemosis score (mean of 24/48/72h and all animals): 0.83 (max. score: 4.0), no data on reversibility Conjunctivae sore (mean of 24/48/72h and all animals): 2.47 (max. score: 3.0), no data on reversibility Iris score (mean of 24/48/72h and all animals): 0.83 (max. score: 2.0), no data on reversibility Cornea opacity score (mean of 24/48/72h and all animals): 1.73 (max. score: 4.0), no data on reversibility No individual scores reported. Other effects: - Significant acute reactions in a minority of animals - overall chemosis and iritis was mild with moderate corneal damage and more severe conjunctival redness (both of the latter still present after 96 h, but	Parent (1992) and Jacobs (1992)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
				showing signs of recovery). No data on negative controls.	
In vivo test; no validated guideline followed. No GLP compliance (study considered reliable with restrictions) Study used principles of the Draize test (1944) for application and assessment of eye effects but measured cornea swelling as an indicator of irritancy to produce a quantitative measure of eye irritancy	**Rabbit**, New Zealand White, Sex: not specified 4 - 6 animals (exact no. not specified)	2-butoxyethanol, (CAS: 111-76-2), (purity: < 97 %)	0.1 mL 100 %, 70 %, 30 %, 20 %, and 10 % test substance/eye (other eye served as control) No washing of eyes. Corneal thickness was measured before instillation and after. Observations: 24, 48, and 72 h, and 7, 10, 14 and 21 days after instillation Vehicle: water 1 Draize score/ observation time (average of the total scores of all rabbits tested). Maximum average score at 24 h post instillation, thus this score was the only one reported.	Eye irritancy ratings according to Texaco single-digit toxicity classification system: minimally irritating: 0-15, slightly irritating: > 15 - 25, moderately irritating: > 25 - 50, severely irritating: > 50 - 80, extremely irritating: > 80 - 110. <u>24 h after instillation</u>: 100 % test material: Draize score of 66.0 (+ 81 % swelling compared to untreated cornea); reversible within 14 days. 70 % test material: Draize score of 49.0 (+ 46 % swelling compared to untreated cornea); no data on reversibility. 30 % test material: Draize score of 39.0 (+14 % swelling compared to untreated cornea); conjunctival damages; no data on reversibility. 20 % test material: Draize score of 2.0 (+ 13 % swelling compared to untreated cornea); no data on reversibility. 10 % test material: Draize score of 1.0 (- 9 % swelling compared to untreated cornea); no data on reversibility. Other effects: 2-butoxyethanol caused significant conjunctival damage as opposed to corneal damage. No data on negative controls.	Kennah et al. (1989)
Ex vivo enucleated rabbit eye test; no validated test guideline followed. GLP compliance not specified	**Rabbit**, New Zealand White, Sex: not specified No. of animals not specified.	2-butoxyethanol, (CAS: 111-76-2), (purity: 99 %)	Clamped eyes placed on superfusion chamber; isotonic saline continuously dripping onto the front part of eye; 0.1 mL undiluted test substance/eye	% swelling: - + 37 % at 0.5 h - + 53 % at 1 h - + 74 % at 2 h - + 113 % at 4 h - + 130 % at 5 h No sign of recovery at 5 h. 60 % swelling was the proposed threshold for classification as irritant.	Jacobs and Martens (1987)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
(study considered reliable with restrictions)			Washing of test substance with saline 10 s after instillation. Observations: 0.5, 1, 2, 4 and 5 h after exposure No controls. Parameter: % swelling		
OECD TG 405, GLP compliant (study considered reliable without restrictions) Deviations from guideline: Not specified	**Rabbit**, New Zealand White, Sex: not specified 3 animals	2-butoxyethanol, (CAS: 111-76-2), (purity: 99 %)	0.1 mL undiluted test substance/eye (other eye served as control) Washing of test substance: not specified, but not assumed Observations: 1, 4, 24, 48 and 72 h, and 4, 7, 9, 10, 12, 14 and 21 days after instillation	Chemosis score (mean of 24/48/72h) of the 3 test animals (max. score: 4.0): 2.3, 3.0, 3.0 1/3 animals still showed a score of 1.0 at day 21. Conjunctivae sore (mean of 24/48/72h) of the 3 test animals (max. score: 3.0): 2.0, 3.0, 2.0 2/3 animals still showed a score of 1.0 at day 21. Iris score (mean of 24/48/72h) of the 3 test animals (max. score: 2.0): 1.0, 2.0, 1.0 Effects fully reversible within 14 days. Cornea opacity score (mean of 24/48/72h) of the 3 test animals (max. score: 4.0): 2.0, 3.0, 2.0 Effects fully reversible within 14 days. No data on negative controls.	ECETOC (1998)
In vitro *(in ovo)* HET-CAM test, according to the ICCVAM-recommended test method protocol GLP compliant (study considered reliable with restrictions) Deviations from ICCVAM	Type of eggs: Fresh, fertilised hen **eggs**. White Leghorn 3 eggs/ group PC: 2 eggs	2-butoxyethanol, (CAS: 111-76-2), (purity: 99.6 %)	0.3 mL of undiluted test substance and 10 % aqueous solution, respectively Exposure period: 210 s Scoring system: 0: no visible change; 1: slight reaction; 2: moderate reaction; 3: severe reaction; Time to reaction was noted.	Undiluted 2-butoxyethanol; effects on the 3 eggs: - Haemorrhagia: 1.0 (after 11 s), 1.0 (after 8 s); 1.0 (after 7 s) - Coagulation: 3.0 (after 17 s); 3.0 (after 28 s); 3.0 (after 25 s) Coagulation: intra- and extravascular. No information on vascular lysis. Conversion of individual scores to appropriate IS-method: IS > 9 → strongly irritating. 10 % 2-butoxyethanol; effects on the 3 eggs: - Haemorrhagia: 1.0 (after 37 s), 1.0 (after 25 s); 1.0 (after 21 s); - Coagulation: 2.0 (after 37 s); 2.0	Anonymous (2004b)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
protocol: Humidity very variable (62.5 ± 7.5 %); no negative control reported; no information on vascular lysis; scoring system different from protocol, but conversion to IS-method possible.			NC: not reported PC: 0.1 N NaOH and 1 % sodium dodecyl sulfate Vehicle: doubly distilled water	(after 30 s); 2.0 (after 33 s) Coagulation: intra- and extravascular. No information on vascular lysis. Conversion of individual scores to appropriate IS-method: IS > 9 → strongly irritating. PC (valid); effects on the 2 eggs: - Haemorrhagia: 2.0 (after 21 s), 2.0 (after 20 s) - Coagulation: 2.0 (after 43 s); 2.0 (after 43 s); Coagulation: only intravascular. No information on vascular lysis. Conversion of individual scores to appropriate IS-method: IS > 9 → strongly irritating.	
In vitro *(in ovo)* HET-CAM test, according to the ICCVAM-recommended test method protocol GLP compliant (study considered reliable with restrictions) Deviations from ICCVAM protocol: Humidity very variable (62.5 ± 7.5 %); no negative control reported; no information on vascular lysis; scoring system different from protocol, but conversion to	Type of eggs: Fresh, fertilised hen **eggs**. White Leghorn 3 eggs/ group PC: 2 eggs	2-butoxyethanol, (CAS: 111-76-2), (purity: 99.68 %)	0.3 mL of undiluted test substance and 10 % aqueous solution, respectively Exposure period: 210 s Scoring system: 0: no visible change; 1: slight reaction; 2: moderate reaction; 3: severe reaction; Time to reaction was noted. NC: not reported PC: 0.1 N NaOH and 1 % sodium dodecyl sulfate Vehicle: doubly distilled water	Undiluted 2-butoxyethanol; effects on the 3 eggs: - Haemorrhagia: 1.0 (after 10 s), 1.0 (after 15 s); 1.0 (after 8 s) - Coagulation: 2.0 (after 42 s); 2.0 (after 28 s); 2.0 (after 25 s) Coagulation: intra- and extravascular. No information on vascular lysis. Conversion of individual scores to appropriate IS-method: IS > 9 → strongly irritating. 10 % 2-butoxyethanol; effects on the 3 eggs: - Haemorrhagia: 1.0 (after 36 s), 1.0 (after 26 s); 1.0 (after 28 s); - Coagulation: 2.0 (after 51 s); 2.0 (after 40 s); 2.0 (after 45 s) Coagulation: intra- and extravascular. No information on vascular lysis. Conversion of individual scores to appropriate IS-method: IS > 9 → strongly irritating. PC (valid); effects on the 2 eggs: - Haemorrhagia: 2.0 (after 21 s), 2.0 (after 20 s) - Coagulation: 2.0 (after 43 s); 2.0 (after 43 s); Coagulation: only intravascular. No information on vascular lysis.	Anonymous (2004a)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
IS(A) and IS(B) method possible.				Conversion of individual scores to appropriate IS-method: IS > 9 → strongly irritating.	
In vivo test; no validated guideline followed (internal BASF test method) No GLP compliance. (study considered reliable with restrictions)	**Rabbit**, Vienna White, Sex: not specified 2 animals	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	1 drop of undiluted test substance/eye (other eye served as control, instilled with physiological solution of sodium chloride) No washing of eyes. Observations: 1, 24, 48, and 72 h, and 8 and 14 days after instillation.	Chemosis score (mean of 24/48/72h and both animals): 2.0 (max. score: 4.0), fully reversible within 8 days. Conjunctivae sore (mean of 24/48/72h and both animals): 2.0 (max. score: 3.0), fully reversible within 8 days. Cornea opacity score (mean of 24/48/72h and both animals): 3.0 (max. score: 4.0), fully reversible within 8 days. No iris score reported. No individual scores reported. No data on negative controls. Other effects: - Sporadically laminar discharge of blood in the nictitating membrane, disappeared within 7 days. - 14 days after instillation, no pathological changes could be found.	BASF AG (1960)
In vivo test; no validated guideline followed (internal BASF test method) No GLP compliance. (study not reliable)	**Rabbit**, Vienna White, Sex: not specified 2 animals	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	1 drop of undiluted test substance/eye (other eye served as control, instilled with physiological solution of sodium chloride) No washing of eyes. Observations: 1, 24 h, and 8 days after instillation.	Chemosis score of both animals for the 3 observation time points (max. score: 4.0): 1 h: 1.0; 4.0 24 h: 1.0; 0 8 d: 1.0; 0 Effects fully reversible within 8 days. Conjunctivae sore of both animals for the 3 observation time points (max. score: 3.0): 1 h: 0; 0 24 h: 0; 0 8 d: 0; 0 Iris score of both animals for the 3 observation time points (max. score: 2.0): 1 h: 0; 0 24 h: 1.0; 0 8 d: 1.0; 0 Effects not fully reversible within 8 days. Cornea opacity score (mean of both animals) for the 3 observation time points (max. score: 4.0):	Anonymous (1968)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
				1 h: 2.0; 1.0 24 h: 1.0; 1.0 8 d: 2.0; 1.0 Effects not fully reversible within 8 days. No data on negative controls. No data for 48 and 72 h reported. Thus, insufficient data for evaluation against the CLP criteria for classification.	
In vivo test; no validated guideline followed (internal BASF test method) No GLP compliance. (study not reliable) Grading of irritation not according to any known (current or former) system (1 - 10)	**Rabbit**, Strain not specified Sex not specified No. of animals not specified	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	0.005 - 0.5 mL (as required) of 1, 5, 15, 40, and 100% test substance/eye Controls: not specified	- 0.005 mL 100% 2-butoxyethanol: severe corneal injuries with iritis. - 0.5 mL of 15 % aqueous dilution of 2-butoxyethanol: moderate corneal injury. - 0.5 mL of 5 % aqueous dilution of 2-butoxyethanol: no injuries. Method and reporting not adequate enough to evaluate eye irritation/corrosion Insufficient data for quantitative interpretation and classification	Bushy Run Research Center (1980b)
In vivo test; method not specified. no validated guideline followed; No GLP compliance. (study not reliable) Grading of irritation not according to any known (current or former) system	**Rabbit**, Strain not specified Sex not specified No. of animals not specified	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	0.005 – 0.5 mL (as required) of undiluted test substance/eye Lid held shut for 1 min No washing of eye Observations: 18 – 24 h Scoring: up to 20 based on combination of corneal effects, iritis, and level of necrosis.	- 0.5 mL 2-butoxyethanol: score > 5.0 (max. score: 20) - 0.2 mL 2-butoxyethanol: score < 5.0 (max. score: 20) No data on reversibility. Method and reporting not adequate enough to evaluate eye irritation/corrosion Insufficient data for quantitative interpretation and classification	Carpenter and Smyth (1946)
In vivo test; Draize	**Rabbit**; Sex and	2-butoxyethanol,	0.1 mL 5 % test substance/eye	Maximum average score: 2.7;	Bagley et al. (1994)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
method; no current validated guideline followed. GLP compliance not specified (study considered reliable with restrictions) Deviations from Draize protocol: no.	strain not specified 4-6 animals	(CAS: 111-76-2), (purity unknown)	(other eye served as control) No washing of eyes. Observations: 1, 24, 48, and 72 h after instillation. When persistent effects, scoring at 4, 7, 10, 12, 14, 16, 18, 21, 28, and 35 days after instillation.	Median days to clear: 1.0. Insufficient data for quantitative interpretation and classification.	
OECD TG 405, GLP compliant (study reliable without restrictions) Deviations from guideline: Not specified.	**Rabbit**, New Zealand White, Sex: not specified 6 animals	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	0.1 mL non-diluted test substance/eye (other eye served as control) No washing of eyes Observations: 1, 24, 48 and 72 h, and 4, 7, 14, and 21 days after instillation	Chemosis score (mean of 24/48/72h) of the 6 test animals (max. score: 4.0): 2.0; 2.0; 2.0; 2.0; 2.0; 1.7 1/6 animals (#2) still showed a score of 2.0 at day 14 and was sacrificed after observation. Conjunctivae sore (mean of 24/48/72h) of the 6 test animals (max. score: 3.0): 2.0; 2.0; 2.0; 2.0; 2.0; 2.0 1/6 animals (#2) still showed a score of 2.0 at day 14 and was sacrificed after observation. Iris score (mean of 24/48/72h) of the 6 test animals (max. score: 2.0): 1.0; 1.0; 1.0; 1.0; 1.0; 0.7 1/6 animals (#2) still showed a score of 1.0 at day 14 and was sacrificed after observation. Cornea opacity score (mean of 24/48/72h) of the 6 test animals (max. score: 4.0): 1.0; 1.0; 1.0; 1.3; 1.3; 1.0 1/6 animals (#2) still showed a score of 2.0 (vascularisation) at day 14 and was sacrificed after observation. 1/6 animals still showed a score of 2.0 (vascularisation) at day 21. Other effects: - Petechial haemorrhage in some animals at 1 to 72 h. - ectropion in some animals from 72 h; not reversible in 1animal at 21 d	Safepharm laboratories (1994b)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
				- one animal (#2) showed signs of discomfort was therefore killed at day 14 No data on negative controls.	
In vivo test; method not specified (internal BASF test); no validated guideline followed. No GLP compliance. (study not assignable)	**Rabbit**; Sex and strain not specified No. of animals not specified	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	1 drop of undiluted test substance/eye (other eye served as control, instilled with physiological solution of sodium chloride) No washing of eyes. Observations: 1, 24 h, and 8 days after instillation.	Only result reported: not irritating. Insufficient data for quantitative interpretation and classification Method and reporting not adequate enough to evaluate skin irritation	BASF AG (1956)
OECD TG 405 (version 1981), GLP compliant (study reliable with restrictions) Deviations from guideline: Observation period only 96 h; control data not reported.	**Rabbit**, New Zealand White, Sex: not specified 6 animals	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	0.1 mL non-diluted test substance/eye (other eye served as control) No washing of eyes Observations: 1, 24, 48 and 72, and 96 h after instillation.	Chemosis score (mean of 24/48/72h and all 6 animals): 0.83 (max. score: 4.0); score of 1.6 after 96 h. Conjunctivae sore (mean of 24/48/72h and all 6 animals): 2.47 (max. score: 3.0); score of 0.2 after 96 h. Iritis score (mean of 24/48/72h and all 6 animals): 0.83 (max. score: 2.0); score of 0.2 after 96 h. Cornea opacity score (mean of 24/48/72h and all 6 animals): 1.73 (max. score: 4.0); score of 1.2 after 96 h. No individual scores reported. Other effects: - Surface of corneal damage after 96 h: 23 % - No data on reversibility within 21 days. No data on negative controls.	Jacobs (1992)
In vivo test; method not specified; no validated guideline followed. No GLP compliance.	**Rabbit**; Sex and strain not specified No. of animals not specified	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	1 drop of 100, 10, or 1 % test substance/eye Observations: 1, 3, 7, and 24 h after treatment.	Undiluted 2-butoxyethanol: - Corneal and conjunctival injuries - Severe redness and chemosis, corneal opacification 10 % 2-butoxyethanol: - Slight conjunctival redness	Hoechst A. (1966)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
(study not assignable)				1 % 2-butoxyethanol: - no effects Insufficient data for quantitative interpretation and classification	
In vitro HET-CAM test, according to the ICCVAM-recommended test method protocol, GLP compliant (study considered reliable with restrictions) Deviations from protocols: No	HET-CAM: Type of eggs: Fresh, fertilised hen **eggs**. White Leghorn 6 eggs/ group Draize:	2-butoxyethanol, (CAS: 111-76-2), (purity: 99.68 %)	0.3 mL of undiluted test substance and 10 % aqueous solution, respectively Exposure period: 300 s Controls: not reported.	Undiluted 2-butoxyethanol: strong irritator No specific data reported. Insufficient data for quantitative interpretation and classification Method and reporting not adequate enough to evaluate skin irritation	Kalweit et al. (1990)
In vivo test; method not specified; no validated guideline followed. No GLP compliance. (study not assignable)	**Rabbit**; Sex and strain not specified No. of animals not specified	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	1 drop of undiluted test substance/ eye; No further information	Only result reported: absence of corneal reflex after treatment. Insufficient data for quantitative interpretation and classification Method and reporting not adequate enough to evaluate skin irritation	Von Oettingen and Jirouch (1931)
In vivo test; method not specified; no validated guideline followed. GLP compliance not specified (study not assignable)	**Rabbit**; Sex and strain not specified 1 animal	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	0.1 mL undiluted test substance/ eye; no further information	- Severe conjunctivitis, iritis, and corneal opacity - Irritation still obvious 21 days after exposure Insufficient data for quantitative interpretation and classification Method and reporting not adequate enough to evaluate skin irritation	Dow Chemical Co. (1981)
In vivo test; method not specified;	**Rabbit**, New Zealand	2-butoxyethanol, (CAS: 111-76-	0.1 mL undiluted test substance/eye (other eye served	Severe eye irritation: - Moderate-to-extensive	Rohm and Haas Co. (1989)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
no validated guideline followed. GLP compliance not specified (study not assignable)	White, Sex: not specified 6 animals	2), (purity unknown)	as control) Washing not specified Observations: not specified	conjunctivitis - Moderate corneal damage - Slight iritis Insufficient data for quantitative interpretation and classification Method and reporting not adequate enough to evaluate skin irritation	
In vivo test; method not specified; no validated guideline followed. GLP compliance not specified (study not assignable)	**Species not specified**; Sex: not specified No. of animals not specified.	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	Dose levels and exposure duration not specified.	Undiluted 2-butoxyethanol: severe ocular irritation 15 % 2-butoxyethanol: moderate corneal injury 5 % 2-butoxyethanol: no corneal injury Insufficient data for quantitative interpretation and classification Method and reporting not adequate enough to evaluate skin irritation	Andersen (1996)

Table 19: Summary table of human data on serious eye damage/eye irritation

Type of data/report	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Repeated inhalation study with humans; Study report	2-butoxyethanol, (CAS: 111-76-2), (purity unknown)	Inhalative exposure to 200 ppm 2-butoxyethanol No. of subjects: 3; Exposure duration: up to 4 h	Immediate irritation of nose and throat, followed by ocular irritation	Mellon Institute of Industrial Research (1955) and Carpenter et al. (1956)

Table 20: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Inhalation study on rats and rabbits	2-butoxyethanol, (CAS: 111-76-2) (purity unknown)	Pregnant **Fischer 344 rats** and New Zealand white rabbits Inhalative exposure during gestational days 6 – 15 with 25 – 200 ppm (rats) and 100 and 200 ppm (rabbits), respectively	Rats and rabbits: Periocular wetness at all concentrations probably due to direct contact of the eyes with 2-butoxyethanol vapour.	Tyl et al. (1984)
Exposure study	2-butoxyethanol	Female **Fischer 344 rats** (10 ± 12 weeks old);	Bilateral retinal changes:	Nyska et al. (1999)

Type of study/data	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
focussing on ocular thrombosis and retinal degeneration induced by 2-butoxyethanol; publication	(CAS: 111-76-2) (purity: 99 %)	No. of animals/group: 8 Concentration: 250 mg 2-butoxyethanol/5 ml water/kg bw Controls: 5 mL water / kg bw; Exposure treatment: 3 consecutive daily treatments; administration by gavage; Animals were sacrificed by CO2 asphyxiation 2 hr after the last scheduled treatment. Complete necropsy examinations were performed on all rats.	- Multifocal haemorrhages within the retinal pigment epithelium (RPE), the choriocapillaris between the RPE and the photoreceptors, and inner and outer nuclear layers. - Retinal detachment - haemorrhages in the RPE associated with degeneration, exfoliation, and loss of these cells - haemorrhages within photoreceptor layers sometimes associated with minimal polymorphonuclear cell infiltration, - loss of photoreceptor cells - haemorrhages in inner and outer nuclear layers associated with degeneration and loss of cells - occasional eosinophilic, amorphous, PAS-positive fibrin thrombi within blood vessels of the ciliary processes and in the limbus	
Ocular expression of vascular cell adhesion molecule (VCAM-1); publication	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %)	Female **Fischer 344 rats** (11–13 wk old); No. of animals: 4/ group; 8 control animals; Concentration: 250 mg 2-butoxyethanol/5 mL water/ kg bw; Controls: 5 mL water/ kg bw; no treatment; Exposure treatment: 2 -4 consecutive daily treatments; administration by gavage; Animals were sacrificed by CO2 asphyxiation 2 hr after the last scheduled treatment. Eyes were fixed for 24 hr in Davidson's fluid, transferred to 70% alcohol after 24 hr, and processed routinely for histology	Histopathological changes: only in eyes of rats exposed to 3 or 4 daily administrations of 2-butoxyethanol - Alterations in the retina (retinal thrombosis, multifocal haemorrhage, degeneration, necrosis; especially in proximity to capillaries) - Retinal detachment - Degeneration and exfoliation - loss of epithelial and photoreceptor cells - presence of neutrophils - thrombi within blood vessels of the ciliary processes, in the limbus and in the retina	Nyska et al. (2003)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		with paraffin embedding and 5–6-µm sectioning. Immunohistochemistry was performed.		

9.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

Animal studies

There are 6 *in vivo* studies on eye irritation/corrosion in rabbits that were performed according to OECD TG 405 and US-FHSA (CFR) protocols, respectively, and which provide sufficient data for CLP classification and are considered relevant and reliable.

The study conducted by BASF (2000) was performed according to OECD TG 405 and GLP with no deviations from the guideline protocol. The study is, hence, considered relevant and reliable without restrictions. 2-butoxyethanol caused moderate damage to the treated eyes: the mean score of cornea opacity of 24 h to 72 h for all 3 animals was 1.0, 0.67 and 1.0, respectively, with 2/3 animals showing a mean score ≥ 1.0 (24 – 72 h). The conjunctivae score of 24 h to 72 h for all 3 was 2.3, 3.0 and 2.3, respectively, and all animals (3/3) exhibited a mean score ≥ 2 (24 – 72 h). The chemosis score of 24 h to 72 h 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 (24 h to 72 h). 2/3 animals showed a mean chemosis score ≥ 2 (24 – 72 h). All observed effects were reversible within the observation time of the study (21 days). However, in this study eyes of the rabbits were washed out after 24 h of exposure. This method is admittedly in accordance with OECD TG 405 ("At 24 hours a washout may be used if considered appropriate."), especially in respect of animal welfare. However, after removing the test substance by washing, it cannot affect the eyes henceforth in the course of the experiment. Thus, washing of the eyes clearly leads to attenuated and potentially underestimated effects of the test substance. The step of substance removal after exposure and, hence, the potential underestimation of the severity of effects, should thus be accounted for in the evaluation of effects.

4 of the 5 further appropriate and reliable *in vivo* studies were also performed according to OECD TG 405 (Jacobs and Martens, 1987; Parent, 1992; Safepharm laboratories, 1994b; ECETOC, 1998), 3 of the 4 studies were GLP compliant (ECETOC, 1998; Parent, 1992; Safepharm laboratories, 1994b). Moreover, in 2 of the 4 studies performed according to OECD TG 405 (Jacobs and Martens, 1987; Parent, 1992), the observation period was only 4 and 7 days, respectively, and thus no information on reversibility of effects at day 21 after instillation were reported. In the 2 other OECD-conform 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 (CFR title 16, section 1500.42)(Grote, 1979b). 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 5 studies, animals were – as described above – instilled with 0.1 mL undiluted test substance in one eye, while the other served as control. Eyes were not washed out during the test and observations were made at 24, 48, and 72 h after instillation and at (several) further time points.

Parent (1992) calculated a mean cornea opacity score of 1.73 (24/48/72 h), and a mean conjunctivae score of 2.4 (24/48/72 h), 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 24/48/72 h) and in 4/6 animals a mean conjunctivae score of ≥ 2.0 (24/48/72 h) 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 (24/48/72 h) were 2.54 in the first test 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 (24/48/72 h) were 1.0 in the first test 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 trial, however the score of 1.59 (24/48/72 h) gives reason to assume an individual score of ≥ 1 (and < 3) in 2/3 animals. Due to the shortened observation period (7 d), no data on reversibility of effects were available.

ECETOC (1998) and Safepharm laboratories (1994b), both reported individual scores for their tests and, moreover, observed animals for 21 days. In the experiments reported by ECETOC (1998), 3/3 animals scored ≥ 2.0 for chemosis and for conjunctivae (24/48/72 h). Furthermore, 3/3 animals received an iris score, as well as a cornea opacity score ≥ 1.0 (24/48/72 h). One animal scored > 1.5 for iritis and > 3 for cornea opacity (24/48/72 h). Conjunctivae effects (1/3 animals) and chemosis 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 a study regarding eye irritation/corrosion in 6 rabbits according to an US-FHSA protocol (no GLP), which is similar to the OECD TG 405, but observation duration is only 7 days and the Draize scoring is applied. Grote (1979b) obtained a chemosis and conjunctivae score ≥ 2 for all (6/6; 24/48/72 h) animals and a cornea opacity score ≥ 1 (and < 3) in 4/6 animals (24/48/72 h), 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 three *in vitro* and one *ex vivo* study available (Anonymous, 2004a; Anonymous, 2004b; Jacobs and Martens, 1987; Kalweit et al., 1990). Two of them were performed according to an accepted and validated test guideline and provided sufficient data for classification (Anonymous, 2004a; Anonymous, 2004b). Both appropriate studies followed the ICCVAM-recommended HET-CAM protocol and are GLP compliant and are considered relevant and reliable. PCs 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 (Anonymous, 2004a; Anonymous, 2004b) yielded in a positive result (IS > 9) for 100 % and 10 % 2-butoxyethanol, indicating that this substance is a strong irritant.

A number of further *in vivo* eye irritation/corrosion studies for 2-butoxyethanol in rabbits are available, but many of these studies were not performed according to any current validated test guideline, are not assignable and/or do not provide sufficient data for quantitative interpretation and indisputable classification according to CLP regulations (Anonymous, 1968; Andersen, 1996; Bagley et al., 1994; BASF AG, 1956; BASF AG, 1960; Bushy Run Research Center, 1980b; Carpenter and Smyth, 1946; Dow Chemical Co., 1981; Hoechst A., 1966; Jacobs and Martens, 1987; Jacobs, 1992; Kennah et al., 1989; Rohm and Haas Co., 1989; Von Oettingen and Jirouch, 1931).

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 hour period of non-exposure (Carpenter et al., 1956). Immediate irritation of the nose and throat, followed by ocular irritation and disturbed taste was reported by all three subjects, potentially due direct contact with 2-butoxyethanol vapour. Whether such 'irritation' was physiological or merely discomfort is not clear.

9.5.2 Comparison with the CLP criteria

Substances that have the potential to seriously damage the eyes are classified in Category 1 (irreversible effects on the eye). Substances that have the potential to induce reversible eye irritation are classified in Category 2 (irritating to eyes).

According to Table 3.3.1 of the CLP Regulation 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 of 3 tested animals, a positive response of: corneal opacity ≥ 3 and/or iritis > 1.5 (calculated as the mean score following grading at 24, 48, 72 hours after installation of the test material)

According to Table 3.3.2 of the CLP Regulation 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 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.

Although the reliable *in vivo* studies mentioned in section 9.5.1 only describe effects which are in accordance with a classification of 2-butoxyethanol as Eye Irrit. 2, in two studies some crucial effects were not reversible within 21 days (chemosis and redness: ECETOC, 1998; cornea opacity: Safepharm, 1994 b). Thus, based on these data, it can be concluded that one criterion for serious eye damage (Eye Dam. 1) given in table 3.3.2 in the CLP Regulation ("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") is fulfilled for 2-butoxyethanol.

Moreover, the ICCVAM considers a substance as causing serious eye damage (Category 1) based on positive results in the HET-CAM test, although the OECD TG 405 assesses each specific major eye structure endpoints up to 21 days post exposure. It should be noted that the HET-CAM test method uses a scoring system and formula to evaluate the degree of blood vessel haemorrhage, lysis, and coagulation. Nevertheless, due to the reliable results of the above mentioned *in vivo* tests performed according to OECD TG 405, the positive results of the two HET-CAM *in vitro* tests (Anonymous,

2004a; Anonymous, 2004b) further support the classification of 2-butoxyethanol as Eye Dam. 1, especially because a 10 % aqueous solution of this substance resulted in a positive test outcome.

Additionally, the report of ocular irritation in humans after temporal inhalation of 2-butoxyethanol and potential direct contact with 2-butoxyethanol vapour (Carpenter et al., 1956), further supports the classification of this substance as Eye Dam. 1.Conclusion on classification and labelling for serious eye damage/eye irritation

According to CLP 2-butoxyethanol has to be classified as:

Eye Dam. 1 and labelled with hazard statement H318: "Causes serious eye damage.", with the pictogram "GHS05: Corrosion", and with the signal word "Danger".

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 ≥ 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 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.**

[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.

9.6 Specific target organ toxicity-repeated exposure

Table 21: Summary table of animal studies on STOT RE. The study documentation is organised regarding route of application (always: oral < inhalation < dermal < other routs), species (always: rats < mice < other species) and study duration (ascending).
The derived LOAEL and (if applicable) NOAEL values of the study results are reported, as well as derived LOAEL values specifically related to criteria on haemolytic anaemia and the corresponding CLP criteria and classification (Cat. 1, Cat. 2 or No classification). To extrapolate equivalent guidance values for toxicity studies of greater or lesser duration than 90 days, dose/exposure time extrapolation similar to Haber's rule for inhalation, which essentially states that the effective dose is directly proportional to the exposure concentration and the duration of exposure, is used (for details see Guidance on the Application of the CLP Criteria, version 4.1). For a 28-day study, for instance, the guidance values are increased by a factor of three. According to the CLP guidance, threshold values for studies ≤ 9 days are extrapolated to 10-times the default guidance value. Haemolytic anaemia is associated with consistent changes in haematology, indicating severe organ dysfunction and/or changes in organ morphology and/or consistent adverse changes in haematology, according to the CLP regulation. Minor effects of low severity or without toxicological significance are not considered. N/A - not applicable.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
Oral				
Haematotoxicity study, *in vivo* No TG followed No GLP compliance (study considered reliable with restrictions) Male and female **Fischer 344 rats** 5-8/group, 10-12/controls	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %) Oral exposure by gavage Vehicle: distilled water (5 mL/kg) Exposure dose/conc.: 250 mg/kg bw/day Exposure duration: daily for 1, 2 or 3 days Post exposure period: 24	No mortality. Haematotoxicity (time-dependent) in both sexes: - decreased RBC count (up to - 80 % in both sexes) - decreased Hb concentration (up to - 21 % in both sexes) - decreased HCT (up to 3-fold in in both sexes) - increase in MCV (up to + 58 % in females and + 56 % in males) - increase in MCH (up to + 8 % both sexes) - decrease in MCHC (up to + 33 % in both sexes) - increase in number of reticulocytes (up to 4-fold in females and 3-fold in males)	250 mg/kg bw/day - CLP criteria, Cat. 2, study duration 1-3 days: 100 < C ≤ 1000 mg/kg bw/day - STOT RE Cat. 2	Ghanayem et al. (2001)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
	or 48 h after last dose.	Morphological changes of erythrocytes: stomatocytosis, macrocytosis with moderate rouleaux formation, and spherocytosis (first observed in females 24 h after first dose). Occasional occurrence of schistocytes and ghost cells, rouleaux formation in both sexes. Morphological changes became progressively more severe as dosing continued. Faster onset and more severe haemolysis in females. Pathological changes (in females mostly after 1 dose, in males after 2 - 3 days of exposure): - Thrombosis in lungs, nasal cavity, eyes, liver, heart, bones and teeth, - Infarction/necrosis in liver, kidneys, heart, eyes, teeth and bones (incl. bone marrow) - Increased spleen with haematopoiesis - Haemoglobinuric nephrosis and splenic extramedullary haematopoiesis. - Erythroid hyperplasia in bones LOAEL: 250 mg/kg bw/day.		
Haematotoxicity study, *in vivo* No TG followed No GLP compliance (study considered reliable with restrictions) Female **Fischer 344 rats**	2-butoxyethanol (CAS: 111-76-2) (purity: 99 %) Oral exposure by gavage Vehicle: water (5 mL/kg) Exposure dose/conc.: 250 mg 2-butoxyethanol/5 ml water/kg bw	No mortality. Bilateral retinal changes: - Multifocal haemorrhages within retinal pigment epithelium (RPE), choriocapillaris, and inner and outer nuclear layers. - Retinal detachment - haemorrhages in the RPE associated with degeneration, exfoliation, and loss of these cells - haemorrhages within photoreceptor layers sometimes associated with minimal polymorphonuclear cell	250 mg/kg bw/day - CLP criteria, Cat. 2, study duration 3 days: $100 < C \leq 1000$ mg/kg bw/day - STOT RE Cat. 2	Nyska et al. (1999)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
8/group (eyes examined only)	Exposure duration: daily for 3 days Post exposure period: 2 h	infiltration, - loss of photoreceptor cells - haemorrhages in inner and outer nuclear layers associated with degeneration and loss of cells - occasional eosinophilic, amorphous, PAS-positive fibrin thrombi within blood vessels of the ciliary processes and in the limbus LOAEL: 250 mg/kg bw/day.		
OECD TG 414 (Prenatal developmental toxicity study) GLP compliant Deviations from TG: dosing not for whole gestation period, 2 tests. (study considered reliable without restrictions) Female **Fischer 344 rats** Test 1: 45- 47/group Test 2: 51 – 58/group 298 total, 104 rats served as controls.	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %) Oral exposure by gavage Vehicle: distilled water (5 mL/kg) Exposure doses/conc.: Test 1: 0, 30, 100, 200 mg/kg bw/day Test 2: 0, 30, 100, 300 mg/kg bw/day Exposure duration: daily Test 1: 3 days (gestational days (GD) 9 – 11) Test 2: 3 days (GD 11 – 13) Post exposure period: 17 days	Details on maternal toxic effects: No mortality. Reduction in body weight and increased spleen weights at ≥ 100 mg/kg bw/day in both studies. Increased kidney and liver weight at ≥ 200 mg/kg bw/day Haematotoxicity: Severe haematotoxicity at ≥ 100 mg/kg bw/day. Dramatic reductions in circulating RBC, HTC, MCHC and Hb concentration after 24 h (haemolytic anaemia). Increases in MCV, MCHC, reticulocytes and white blood cell count. By GD 20 the haematotoxic effects were nearly reversed. No quantitative details reported. No information on erythrocyte morphology. LOAEL: 100 mg/kg bw/day. NOAEL: 30 mg/kg bw/day. Details on embryotoxic / teratogenic effects: Decreased fetal blood platelet count at 300 mg/kg bw/day dosed on GD 11 – 13 (NOAEL: 100 mg/kg bw/day).	100 mg/kg bw/day - CLP criteria, Cat. 2, study duration 3 days: 100 < C ≤ 1000 mg/kg bw/day - STOT RE Cat. 2	Sleet et al. (1991)

Haematotoxicity study, *in vivo* No TG followed No GLP compliance (study considered reliable with restrictions) Female **Fischer 344 rats** 4/group; 8/controls Eyes of rats were studied histologically and immunohistochemically (e.g. for expression of vascular cell adhesion molecule-1 (VCAM-1).	2-butoxyethanol (CAS: 111-76-2) (purity: 100 %) Oral exposure by gavage Vehicle: drinking water Exposure dose/conc.: 250 mg/kg bw/day Exposure duration: daily for 2, 3 or 4 days Sacrifice of the animals 2 h after last treatment.	No mortality. Positive VCAM-1 expression in eyes of rats exposed to 3 and 4 doses (in iris (epithelium lining the posterior surface, anterior mesenchymal epithelium), ciliary processes (lining epithelium, stromal cells), and retina (hypertrophic retinal pigment epithelium)). Weak immunolabeling in eyes exposed to only 2 doses. (VCAM-1are membrane glycoproteins functionally important for the adhesion of erythrocytes and leukocytes to activated endothelium.) Appearance of VCAM-1 immunostaining correlated with development of thrombosis in the same structures. Retina lesions: Retinal thrombosis associated with degeneration and loss of cells resulting in thinning, disorganisation, and fusion of these layers; Multifocal haemorrhage associated with degeneration, exfoliation, loss of epithelial cells and presence of neutrophils within the retinal pigment epithelium leading to disorganisation, and fusion of these layers; Necrosis (mainly in proximity to capillaries). Haemorrhage caused organizational disruption and loss of photoreceptor cells. Thrombi within blood vessels of the ciliary processes, in the limbus and in the retina. Conclusions: VCAM-1 functions in the pathogenesis of 2-butoxyethanol-related thrombosis by promoting adhesion of erythrocytes to the endothelium. LOAEL: 250 mg/kg bw/day.	250 mg/kg bw/day - CLP criteria, Cat. 2, study duration 2-4 days: $100 < C \leq 1000$ mg/kg bw/day - STOT RE Cat. 2	Nyska et al. (2003)
Haematotoxicity study, *in vivo* No TG followed No GLP compliance (study considered reliable with restrictions)	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %) Oral exposure by gavage Vehicle: distilled water (5 mL/kg) Exposure dose/conc.: 250	No mortality. No effect on RBC aggregability. Inconclusive effects on RBC deformability. Increase in RBC adherence to extracellular matrix (EC; no differences between the sexes). Highest adherence at day 2,	N/A	Koshkaryev et al. (2003)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
Male and female **Fischer 344 rats** 4/group (blood examined only)	mg/kg bw/day Exposure duration: daily for 2, 3 or 4 days Collection of blood samples 2 h after exposure (group 1) and before sacrifice 24 days after last dosing (group 2).	then sharp decrease with time. RBC-EC-interactions have been shown to be a potent catalyst of vascular occlusion in haemolytic haemoglobinopathies. The enhanced RBC adherence to EC could be a mechanism by which thrombosis and organ infarct are induced in 2 -butoxyethanol treated rats.		
Haematotoxicity study, *in vivo* No TG followed No GLP compliance (study considered reliable with restrictions) Male **Fischer 344 rats** 24/group	2-butoxyethanol (CAS: 111-76-2) (purity: 99.9 %) Oral exposure by gavage Vehicle: distilled water (5 mL/kg) Exposure doses/conc.: 0, 500, 1000 mg/kg bw/day Exposure duration: daily for 4 days Animals sacrificed at days 1, 4, 8, 22 post exposure (6 per time point).	No mortality. Reduced body weight gain at 1000 mg/kg bw/day. Increased spleen, liver and kidney weight (no reversibility within study period). Decreased thymus weight (returned to normal by day 22). Splenic extramedullary haematopoiesis at both concentrations at day 1, returned to normal by day 8. Transient lymphocyte depletion of the thymic cortex at both concentrations, returned to normal by day 4. Haematotoxicity: - reduced RBC count (day 1 after exposure: - 23 % at 500 mg/kg bw/day; - 49 % at 1000 mg/kg bw/day; day 8 after exposure: - 10 % at 500 mg/kg bw/day; - 11 % at 1000 mg/kg bw/day). Full recovery 22 days after exposure. - reduced HCT (day 1 after exposure: - 22 % at 1000 mg/kg bw/day). Full recovery 4 days after exposure. - reduced Hb concentration (day 1 after exposure: - 33 % at 1000 mg/kg bw/day; day 4 after exposure: full recovery at 500 mg/kg bw/day; - 6 % at 1000 mg/kg bw/day). Full recovery 8 days after exposure.	500 mg/kg bw/day - CLP criteria, Cat. 2, study duration 4 days: 100 < C ≤ 1000 mg/kg bw/day - STOT RE Cat. 2	Grant et al. (1985)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
		- elevated MCV (day 1 after exposure: + 24 % at 500 mg/kg bw/day; + 37 % at 1000 mg/kg bw/day; day 22 after exposure: full recovery at 500 mg/kg bw/day; + 3 % at 1000 mg/kg bw/day). - increased reticulocytes counts (day 1 after exposure: ~ 6-fold at 500 mg/kg bw/day; ~ 7-fold at 1000 mg/kg bw/day). Full recovery 8 days after exposure. - increased MCH (day 1 after exposure: + 16 % at 500 mg/kg bw/day; + 32 % at 1000 mg/kg bw/day; 22 days after exposure: + 5 % at 500 mg/kg bw/day; + 7 % at 1000 mg/kg bw/day). No recovery during study period. - increased number of normoblastes, pronounced anisocytosis, polychromasia and presence of Howell Jolly bodies at 1000 mg/kg bw/day, resorbed by day 8. Depression of leucocytes throughout the study, due to a decreased number of circulating lymphocytes at both concentrations (up to - 70 %). Values gradually increased after exposure, but did not reach control values by the end of the recovery period. LOAEL: 500 mg/kg bw/day.		
Haematotoxicity study, *in vivo* No TG followed No GLP compliance (study considered reliable with restrictions) Female **Fischer 344 rats**	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %) Oral exposure by gavage Vehicle: drinking water (5 mL/kg) Exposure doses/conc.: 250 mg/kg bw/day Exposure duration: daily	No mortality. Haematotoxicity: - acute regenerative haemolytic anemia - significant decrease in RBC counts (- 75 %) - significant increase in MCV (+ 54 %) - significant decrease in Hb concentration (no details reported) - significant decrease in HCT (no details reported) - erythrocytes: macrocytosis, moderate poikilocytosis with	250 mg/kg bw/day - CLP criteria, Cat. 2, study duration 4 days: 100 < C ≤ 1000 mg/kg bw/day -	Lewis et al. (2006)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
5-6/group	for 4 days	stomatocytes and schistocytes, as well as moderate polychromatophilia Histopathology: - significant thromboses in nasal cavity, incisor teeth, coccygeal vertebrae, femur, liver, and lungs LOAEL: 250 mg/kg bw/day.	STOT RE Cat. 2	
Haematotoxicity study, *in vivo* No TG followed No GLP compliance (study considered reliable with restrictions) Female **Fischer 344 rats** (6-weeks old and 12-weeks old) 5/age/group (blood, ICAM-1expression and histology of various organs examined)	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %) Oral exposure by gavage Vehicle: tap water (5 mL/kg) Exposure doses/conc.: 62.5, 125, and 250 mg/kg bw/ day Exposure duration: daily for 2, 3 or 4 days Post exposure period: 2 h	No mortality. Haematotoxicity: - significant decrease in RBC counts at ≥ 125 mg/kg bw/day in 6-week-old rats and ≥ 62.5 mg/kg bw/day in 12-week-old rats (> 10 % at 125 mg/ kg bw/day and max. - 13 % in 6-week old rats; > 10 % at 62.5 mg/kg bw/day, max. - 29 % in 12-week old rats) - significant increase in MCV at both ages at and ≥ 62.5 mg/kg bw/day (> 10 % at 62.5 mg/kg bw/day in 6- and 12-week old rats; max. - 11 and - 24 % in 6- and 12-week-old rats, respectively) - significantly increased endothelial intercellular adhesion molecule-1 (ICAM-1) at ≥ 125 mg/kg bw/ day in 6-week-old rats and at ≥ 62.5 mg/kg bw/ day in 12- week-old rats Significant increase in relative spleen at 250 mg/kg bw/day in 6-week-old rats. Pale liver, and pink-red discoloration of distal aspect of the tail most frequently at 250 mg/kg bw/day in 12-week-old rats. Histopathology: - intravascular thrombi in any of the 6-week-old animals, but in 12-week-old rats at 250 mg/kg bw/day, most prominent	125 mg/kg bw/day - CLP criteria, Cat. 2, study duration 2-4 days: 100 < C ≤ 1000 mg/kg bw/day - STOT RE Cat. 2	Ramot et al. (2007)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
		after 4 days of treatment - most thrombi in tail, nasal cavity, incisor teeth, and bone marrow in 12-week-old rats at ≥ 125 mg/kg bw/day - liver lesions in 12-week-old rats at ≥ 125 mg/kg bw/day (multifocal hepatocellular necrosis) - thrombi also in the ciliary-body capillaries of the eye, auricle of the heart, and choroid plexus of the brain - intracapillary microthrombi in renal glomeruli at 250 mg/kg bw/day - interstitial vessels in kidneys at 250 mg/kg bw/day - splenic extramedullary haematopoiesis in 6- and 12-week-old animals at 250 mg/kg bw/day - renal intratubular haemoglobin crystals in the 12-week-old animals at 250 mg/kg bw/day LOAEL: 62.5 mg/kg bw/day in both age groups.		
Haematotoxicity study, *in vivo* No TG followed No GLP compliance (study considered reliable with restrictions) Male and female **Fischer 344 rats** 4/sex/group	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %) Oral exposure by gavage Vehicle: water (5 mL/kg) Exposure doses/conc.: 250 mg/kg bw/day Exposure duration: daily for 1, 2, 3 or 4 days Post exposure period: 28 days	No mortality. Piloerection, hutched posture, bloody urine, decreased spontaneous motor activity 8 h after exposure. Apathy during 3 consecutive dosing days (recovery after 28 days post exposure). Significant decrease in body weight (- 7 – 14 %). Significant increase in absolute and relative spleen weights in both sexes. Haematotoxicity (no quantitative details reported): - significantly decreased RBC counts (up to - 30 %) - significant decrease in Hb conc. (up to - 28 %) - significant decrease in HCT (up to - 48 %) - significant decrease in MCHC (up to - 62 %) - significant increase in MCH (up to + 17 %) - significant increase in MCV (up to + 83 %)	250 mg/kg bw/day - CLP criteria, Cat. 2, study duration 2-4 days: 100 < C ≤ 1000 mg/kg bw/day - STOT RE Cat. 2	Ezov et al. (2002)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
		- anisocytosis of erythrocytes: macrocytosis, schistocytosis and severe hypochromic ghost cells (linear dose-response) - significant increase in no. of nucleated RBC prematurely released from bone marrow (linear dose/time-response) - stomatocytes - hypochromic RBC - increase in no. of polychromatophylic RBC and reticulocytes - significant thrombocytosis (incl. platelet clumps and rouleaux formation) Females more sensitive, effects more pronounced. Pathological effects: - dark spleen, kidneys - pale liver - thrombosis (both sexes: coccygeal, vertebrae, heart; females only: femur, brain, liver, lungs, eyes) and infarction - renal tubular necrosis associated with haemoglobin casts (haemoglobinuric necrosis) - splenic extramedullary haematopoiesis LOAEL: 250 mg/kg bw/day.		
Haematotoxicity study, *in vivo* No TG followed No GLP compliance (study considered reliable with restrictions) Male and female **Fischer**	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %) Oral exposure by gavage Vehicle: water (5 mL/kg) Exposure doses/conc.: 250 mg/kg bw/day Exposure duration: 1, 2, 3,	No mortality. Congested and dilated blood vessels, presence of vascular occlusive thrombi in pulp of incisor and molar teeth (time-dependent increase in severity). Progressive necrosis of odontoblasts with well-defined border between necrotic and vital cells in females. At day 29, all changes disappeared completely, normal tissue appearance. Focal myocytic necrosis of tongue at day 2-4 with	250 mg/kg bw/day - CLP criteria, Cat. 2, study duration 2-4 days: $100 < C \leq 1000$ mg/kg bw/day -	Redlich et al. (2004)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
344 rats 4/sex/group Teeth, tongue and dental pulp examined only)	4 days Post exposure period: 2 h, one group after 24 days	regenerative changes. More severe in females than males. Normal tongue in both sexes at day 29.	STOT RE Cat. 2	
Haematotoxicity study, *in vivo* No TG followed No GLP compliance (study not assignable) Male and female Fischer 344 rats 4/sex/group	2-butoxyethanol (CAS: 111-76-2) (purity unknown) Oral exposure Vehicle: water (5 mL/kg) Exposure doses/conc.: 250 mg/kg bw/day Exposure duration: daily for 4 days	No mortality. Thrombosis and infarction of tail vertebrae in both sexes, females more severely affected. Lesions characterized by extensive medullary fat necrosis, granulomatous inflammation, fibroplasia, growth plate degeneration, and new woven bone formation adjacent to necrotic bone trabeculae.	250 mg/kg bw/day - CLP criteria, Cat. 2, study duration 4 days: $100 < C \leq 1000$ mg/kg bw/day - STOT RE Cat. 2	Shabat et al. (2004)
Haematotoxicity study, *in vivo* No TG followed No GLP compliance (study considered reliable with restrictions) Female **Sprague Dawley rats** 12/group; 3 groups	2-butoxyethanol (CAS: 111-76-2) (purity: 99 %) Oral exposure by gavage Vehicle: water (5 mL/kg) Exposure dose/conc.: 500 mg/kg bw/day Exposure regimen: daily, 7 days; on day 7 one group and the controls received 1500 mg/kg 2-butoxyethanol (LD50 dose), third group	No mortality. Ataxia, visible haematuria, paleness of skin, piloerection and morbidity during initial exposure, but rapid recovery. Haematotoxicity: HCT decreased to 18.6 24 h after treatment (baseline HCT: ~ 40.0). Recovery by day 7. All rats that received pre-treatment survived the subsequent lethal challenge with 1500 mg/kg of 2-butoxyethanol while the rats that received vehicle only exhibited 90 % mortality. Survivors of control group showed an average HCT of 13.5 whereas pre-treated rats experienced a much lesser decrease in HCT.	500 mg/kg bw/day - CLP criteria, Cat. 2, study duration 7 days: $100 < C \leq 1000$ mg/kg bw/day - STOT RE Cat. 2	Sivarao and Mehendale (1995)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
	received water instead. Post exposure period: 14 days	LOAEL: 500 mg/kg bw/day.		
Haematotoxicity study, *in vivo* No TG followed No GLP compliance (study considered reliable with restrictions) Male **Fischer 344 rats** 6/group	2-butoxyethanol (CAS: 111-76-2) (purity: 99 %) Oral exposure by gavage Vehicle: water Exposure dose/conc.: 125 mg/kg bw/day Exposure regimen: daily for 1, 2, 3, 6 or 12 consecutive days. Post exposure period: 24 h after last dose	No mortality. Time-dependent increase in haemolysis of erythrocytes: - decreased RBC count (- 24 % after 12 days) - decreased Hb concentration (> 10 % after 6 days, - 13 % after 12 days) - decreased HCT (- 49 % after 2 days, but recovered until end of study) - increase in MCV (+ 20 % after 12 days). - increased ATP concentrations and increased number of reticulocytes (linear dose-response up to 6 days), then slowly declined, but remained above control levels throughout the study (ATP after 12 days 29 % higher; no. of reticulocytes after 12 days 57 % higher) Increased relative spleen weight spleen (max. after 6 days of exposure), declined slowly with extended dosing regimen (days 6 – 12). Following a moderate decline on day 3 and 6, spleen weight was increased on day 12 compared (+ 40 %). LOAEL: 125 mg/kg bw/day.	125 mg/kg bw/day - CLP criteria, Cat. 2, study duration **6 days**: 100 < C ≤ 1000 mg/kg bw/day - STOT RE Cat. 2 --------- 125 mg/kg bw/day - CLP criteria, Cat. 2, study duration **12 days**: 75 < C ≤ 750 mg/kg bw/day - STOT RE Cat. 2	Ghanayem et al. (1992)
Haematotoxicity study,	2-butoxyethanol (CAS:	No mortality. Significant lower body weight gain or even body weight loss during study day 1- 15 at ≥ 250 mg/kg	100 mg/kg bw/day	Kenyon et al. (2015)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
in vivo No TG followed No GLP compliance (study considered reliable with restrictions) Male **Wistar-Han IGS rats** 6/group	111-76-2) (purity: 99 %) Oral exposure by gavage Vehicle: water (10 mL/kg) Exposure doses/conc.: 0, 10, 100, 250, 450 mg/kg/day Exposure duration: daily for 28 days Post exposure period: 7 days	bw/day (recovered by day 29). Haematotoxicity: - haemoglobinuria at ≥ 100 mg/kg bw/day (3/6 at lowest dose, 100% at higher doses); recovery within 1 week - significant decrease in RBC count at ≥ 100 mg/kg bw/day after 2 and 8 days of exposure (- 12 % at 100 and - 44 % at 450 mg/kg bw/day, respectively); recovered slowly (still significant at ≥ 250 mg/kg bw/day after 35 days) - Significant decrease in Hb concentration at ≥ 100 mg/kg bw/day after 2 and 8 days of exposure; after 2 days: - 12 % at 100, - 33 % at 250, and - 24 % at 450 mg/kg bw/day, respectively; after 8 days: - 12 % at 100, - 14 % at 250, and - 45 % at 450 mg/kg bw/day, respectively; recovered until day 35 - Significant decrease in HCT at ≥ 100 mg/kg bw/day after 2 and 8 days of exposure (- 11 % at 100 and - 40 % at 450 mg/kg bw/day, respectively); recovered until day 35 - Significant increase in MCV at ≥ 250 mg/kg bw/day until end of study (day 35) LOAEL: 100 mg/kg bw/day. NOAEL: 10 mg/kg bw/day.	- CLP criteria, Cat. 2, study duration **8 days**: 100 < C ≤ 1000 mg/kg bw/day - STOT RE Cat. 2 ---------- 250 mg/kg bw/day - CLP criteria, Cat. 2, study duration **28 days**: 30 < C ≤ 300 mg/kg bw/day - STOT RE Cat. 2	
Haematotoxicity study, *in vivo* No TG followed No GLP compliance (study not assignable) **Wistar rats**	2-butoxyethanol (CAS: 111-76-2) (purity unknown) Oral exposure Vehicle: assumed to be water Exposure doses/conc.: not	No mortality. Marked haematuria, proteinuria, glucosuria, bilirubinuria, and elevated urobilinogen consistent with intravascular haemolysis after 4 and 24h. Urinalysis parameters returned to normal by 8 days. Haematotoxicity: - decreased RBC (- 21 %), depressed throughout the study	N/A	Myler et al. (2004a) and Myler et al. (2004b)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
Sex: not specified No. of animals not specified	specified Exposure duration: daily for 4, 24 h, 8 or 36 days	- increased MCV (+ 33 %) within 4 h - occurrence of stomatocytes - increase in white blood cell count (+ 132%) within 4 h, returned to normal by 8 day - increased aspartate aminotransferase and bilirubin (99% and 2000% over control, respectively) after 4 h after, returned to normal by day 8 - increase in peripheral reticulocytes and polychromatic erythrocytes in bone marrow and spleen throughout the study Increased spleen weight (+ 55 %) after 8 days. Increased haemosiderin pigment associated with activated macrophages in liver and spleen throughout the study. Increased numbers of Kupffer cells (101% over control) at day 36.		
OECD TG 407 (Repeated dose 28-days oral toxicity study in rodents) No GLP compliance Deviations from TG: several endpoints not examined. (study considered reliable with restrictions) Male **CR, COBS, CD,**	2-butoxyethanol (CAS: 111-76-2) (purity: 99.5 %) Oral exposure by gavage Vehicle: water Exposure doses/conc.: 0, 222, 443, 885 mg/kg bw/day Exposure duration: 5 days/week; 6 weeks	2 spontaneous deaths in the high dose group only. Blood in urine at ≥ 443 mg/kg bw/day throughout the study and in 1 rat at 222 mg/kg bw/day after 3 weeks. Lethargy, unkempt hair coats, piloerection, rales, slight weakness and inactivity at ≥ 443 mg/kg bw/day. Significant body weight reduction at 885 mg/kg bw/day after 13 days. Haematotoxicity: Decrease in Hb concentration, total RBC count. Increase in MCHC at all doses (linear dose-response). At ≥ 443 mg/kg bw/day: decreased MCH, increased MCV. No details reported.	N/A (due to lack of sufficiently detailed data)	Eastman Kodak (1982)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
BR albino rats 10/group		Increased spleen weight at ≥ 443 mg/kg bw/day, spleen enlarged and dark . Increased liver weight at ≥ 222 mg/kg bw/day. Splenic congestion and extramedullary haematopoiesis in spleen at all doses. Stomach hyperkeratosis at all doses. Liver lesions at ≥ 443 mg/kg bw/day: heptocytomegally (high dose only), anisokaryosis (low and mid dose) and haemosiderin deposition (high and mid dose). Renal effects: proteinaceous casts and haemosiderin in the proximal convoluted tubules at ≥ 443 mg/kg bw/day. LOAEL: 222 mg/kg bw/day (nominal)		
Haematotoxicity study, *in vivo* No TG followed No GLP compliance (study considered reliable with restrictions) Male **Crl:COBS CD (SD)BR rats** 10/group	2-butoxyethanol (CAS: 111-76-2) (purity: 99 %) Oral exposure of undiluted2-butoxyethanol by gavage Exposure doses/conc.: 222, 443, 885 mg/kg bw/day Exposure duration: 5 days/ week for 6 weeks	No mortality. Significant dose-dependent decrease in body weight gain at 443 and 885 mg/kg bw/day from day 13 onwards. Significant dose-dependent: - decrease in Hb concentration at all dose levels (- 7 % at 222 mg/kg bw/day; - 22 % at 443 mg/kg bw/day; - 22 % at 885 mg/kg bw/day) - decrease in RBC counts at all dose levels (- 12 % at 222 mg/kg bw/day; - 12 % at 443 mg/kg bw/day; - 34 % at 885 mg/kg bw/day) - decrease in MCHC at 443 mg/kg bw/day (- 10 %) and 885 mg/kg bw/day (- 20 %). - increase in MCH at all doses (+ 6 % at 222 mg/kg bw/day; ~+10 % at 443 mg/kg bw/day; + 18 % at 885 mg/kg	222 mg/kg bw/day - CLP criteria, Cat. 2, study duration 42 days: 20 < C ≤ 200 mg/kg bw/day - STOT RE No Classification	Krasavage (1986)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
		bw/day) - increase in mean corpuscular volume at all dose levels (+ 8 % at 222 mg/kg bw/day; + 24 % at 443 mg/kg bw/day; + 48 % at 885 mg/kg bw/day). Blood urine at ≤ 443 mg/kg bw/day throughout the study period. Increased spleen weights, splenic congestion at ≥ 222 mg/kg bw/day. Increased relative liver weights. Hepatocytomegalie and haemosiderin accumulation in liver at ≥ 443 mg/kg bw/day and kidneys at all doses. Increased serum alkaline phosphatase (at 443 and 885 mg/kg bw/day) and serum alanine aminotransferase (at 885 mg/kg bw/day) levels. Significantly reduced glucose levels at 885 mg/kg bw/day. Lethargy, rough hair coats and slight piloerection at 885 mg/kg bw/day. LOAEL: 222 mg/kg bw/day.		
OECD TG 408 (Repeated dose 90-days oral toxicity study in rodents) GLP compliant No deviations from TG (study considered reliable without	2-butoxyethanol (CAS: 111-76-2) (purity: 99 %) Continuous oral exposure via drinking water Exposure doses/conc.: 0, 750, 1500, 3000, 4500, 6000 ppm (equivalent to 0, 82, 151, 304, 363, 470 mg/kg bw/day for females;	No mortality. Reduced body weight (~ 20%) at 4500 and 6000 ppm. Haematotoxicity: - macrocytic and hypochronic anaemia - reduced RBC count at ≥ 750 and ≥ 1500 mg/kg bw/day in females and males, respectively (> 10 % at 3000 ppm after 1 week and at 1500 ppm after 13 weeks) - reduced Hb concentration at ≥ 750 and ≥ 1500 mg/kg	281 and 304 mg/kg bw/day in males and females, respectively (based on reduction (> 10 %) in RBC counts and Hb concentration) -	NTP (1993)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
restrictions) Male and female **Fischer 344 rats** 20/group (no information whether eyes and tail were examined histo-pathologically)	0, 69, 129, 281, 367, 452 mg/kg bw/day for males) Exposure duration: daily for 90 days	bw/day in females and males, respectively (> 10 % at 3000 ppm after 1 week and at 6000 ppm after 13 weeks) - reduced HTC at ≥ 1500 and ≥ 45000 mg/kg bw/day in females and males, respectively - increased reticulocyte counts from week 1 – 13 at ≥ 3000 ppm - thrombocytoapenia at ≥ 4500 ppm at all time points and at 3000 ppm in females at week 13. - Males: decrease in erythrocyte counts at ≥ 3000 ppm. Females: decrease in erythrocyte counts at ≥ 1500 ppm. Pathology: - liver lesions at all doses (linear dose-response): cytoplasmic alteration (at ≥ 750 ppm); eosinophilic, hepatocellular degeneration (at ≥ 3000 ppm); pigmentation (Kupffer cells; at ≥ 1500 ppm). - hyperplasia of bone marrow at ≥ 3000ppm. - increased haematopoiesis and haemosiderin pigmentation in spleen at ≥ 1500ppm (linear dose-response). Females slightly more susceptible. LOAEL: 69.0 and 82.0 mg/kg bw for males and females, respectively. BMD_{10}: 38 mg/kg for both sexes. $BMDL_{10}$: 27 and 20 mg/kg bw/day for males and females, respectively.	CLP criteria, Cat. 2, study duration **7 days**: 100 < C ≤ 1000 mg/kg bw/day - STOT RE Cat. 2 ---------- 129 and 151 mg/kg bw/day in males and females, respectively (based on reduction (> 10 %) in RBC counts) - CLP criteria, Cat. 2, study duration **90 days**: 10 < C ≤ 100 mg/kg bw/day -	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
			STOT RE No Classification	
Haematotoxicity study, *in vivo* No TG followed No GLP compliance (study considered reliable with restrictions) Male **Fischer 344 rats** 20/group (DNA synthesis, oxidative damage, HCT and iron deposition in liver examined only)	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %) Oral exposure by gavage Vehicle: water Exposure doses/conc.: 0, 225, 450 mg/kg Exposure duration: 5 days/week for 7, 14, 28 and 90 days	No mortality. Decrease in body weight at 450 mg/kg after 28 and 90 days. Haematotoxicity: Increased haemolysis: decreased HCT at all time points (approx. - 15 % at both concentrations after 90 days); Significantly higher spleen weight at all doses already after 7 days. Dose and time related significant increase of Perl's index in Kupffer cells (hepatic deposition of iron; 2 to 10-fold at 225 mg/kg and 4 to 25-fold at 450 mg/kg), assumed to indicate haemosiderin deposition following haemolysis. Reduced hepatic vitamin E levels. No changes in DNA synthesis in liver. LOAEL: 225 mg/kg bw/day.	N/A	Siesky et al. (2002)
Developmental toxicity study No TG followed GLP compliance not specified (study considered reliable with restrictions) Female **CD-1 mice**	2-butoxyethanol (CAS: 111-76-2) (purity: 97 %) Oral exposure by gavage Vehicle: distilled water (10 mL/kg) Exposure doses/conc.: Test 1: 0, 350, 650, 1000 and 1500, 200 mg/kg bw/day	Maternal toxic effects: Mortality: 50 % at 1500 mg/kg bw/day; 100 % at 2000 mg/kg bw/day. Significantly reduced body weight at 1500 mg/kg bw/day. General morbidity at ≥ 1500 mg/kg bw/day: lethargy, failure to right, abnormal breathing, and/or cold to the touch. Haematotoxicity: Significant haemolysis at ≥ 650 mg/kg bw/day. No quantitative details reported.	N/A	Wier et al. (1987)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
6/group	Test 2: 0, 30, 100, 300 mg/kg bw/day Exposure duration: daily, 7 days (GD 8 – 14)	NOAEL: 350 mg/kg bw/day.		
Toxicokinetic study No TG followed No GLP compliance (study considered reliable with restrictions) Male and female **B6C3F1 mice** 5/sex/group	2-butoxyethanol (CAS: 111-76-2) (purity unknown) Oral exposure by gavage Vehicle: assumed to be water Exposure by gavage (100 %; no vehicle) Exposure doses/conc.: 100, 400, or 800 mg/kg-day (the 100 mg/kg-day treatment was increased to 1200 mg/kg-day after 2 days) Exposure duration: daily for 1 week Study was terminated after 4 exposure days due to high mortality.	Severe haemolysis and mortality. Forestomach lesions consisting of focal areas of irritation and epithelial hyperplasia at all exposure levels. No details reported.	N/A	Corley et al. (1999)
Haematotoxicity study, *in vivo* No TG followed	2-butoxyethanol (CAS: 111-76-2) (purity: 99 %) Oral exposure by gavage	No mortality. Haematotoxicity after 7 days: - significant decrease in RBC counts (- 23 %) - significant decrease in Hb concentration (- 19 %)	900 mg/kg bw/day - CLP criteria, Cat. 2, study duration 7 days:	Laifenfeld et al. (2010)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
No GLP compliance (study considered reliable with restrictions) Male **B6C3F1 mice** 10/group (Blood, bone marrow (left femur), liver, and spleen examined only)	Vehicle: deionised water Exposure dose/conc.: 900 mg/kg bw/day Exposure regimen: daily, 7 days Post exposure period: 14 days	- significant increase in no. of reticulocytes (6.1-fold) Histopathology: - significantly increased splenic extramedullary haematopoiesis: increased numbers of haematopoietic precursors in the medullary red pulp with a predominance of erythroid lineage cells compared to myeloid precursors - significantly decreased bone marrow myeloid:erythroid (M:E) ratio LOAEL: 900 mg/kg bw/day.	100 < C ≤ 1000 mg/kg bw/day - STOT RE Cat. 2	
Haematotoxicity study, *in vivo* No TG followed GLP compliance not specified (study considered reliable with restrictions) Female **B6C3F1 mice** Test 1: 5/group (all concentrations) Test 2: 10/group (only 500 mg/kg/day)	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %) Oral exposure by gavage Vehicle: not specified, assumed to be water Exposure doses/conc.: 0, 50, 150, 500 mg/kg/day Exposure duration: daily for 10 days Post exposure period: 18 h	No mortality. Marked hyperkeratosis in the forestomach at 500 mg/kg/day. Haematotoxicity at 500 mg/kg/day, single cases observed at 150 mg/kg/day. No details reported. NOAEL: 50 mg/kg/day.	N/A	Green et al. (2002)
Haematotoxicity study, *in vivo* No TG followed No GLP compliance	2-butoxyethanol (CAS: 111-76-2) (purity unknown) Oral exposure	No mortality. Marked haematuria, proteinuria, glucosuria, bilirubinuria, and elevated urobilinogen consistent with intravascular haemolysis after 4 and 24h. Urinalysis parameters returned to normal by 8 days.	N/A	Myler et al. (2004a) and Myler et al. (2004b)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
(study not assignable) **B6C3F1 mice** Sex: not specified No. of animals not specified	Vehicle: assumed to be water Exposure doses/conc.: not specified Exposure duration: daily for 4 h, 24 h, 8 or 36 days	Haematotoxicity (no quantitative details reported): - decrease in RCB counts (- 10 %) within 4 h, remained depressed throughout the study - increased MCV (+ 22 %) within 4 h - occurrence of stomatocytes (abnormal RBC, in which a slit or mouth-like area replaces the normal central circle of pallor) - increase in peripheral reticulocytes and polychromatic erythrocytes in bone marrow and spleen throughout the study Increased white blood cell count (+ 127 %) within 4 h, returned to normal by day 8 Increased aspartate aminotransferase and bilirubin (+ 160% and + 467% over control, respectively) after 4 h, returned to normal by day 8 Increased spleen weight + 75 % over control) after 8 days		
Repeated dose toxicity study No TG followed GLP compliance not specified (study considered reliable with restrictions) Male **JCL-ICR mice** 5/group	2-butoxyethanol (CAS: 111-76-2) (purity unknown) Oral exposure by gavage Exposure doses/conc.: 0, 500, 1000, 2000 mg/kg bw/day Exposure duration: 5 days/ week for 5 weeks	100 % mortality at 2000 mg/kg bw. Haematotoxicity: Decrease in white blood cell counts; toxic effects on leucocytes; reduced RBC count at all doses. No quantitative details reported No/slight effects on MCV or Hb levels. LOAEL: 500 mg/kg bw/day	N/A	Nagano et al. (1984)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
Haematotoxicity study, *in vivo* No TG followed No GLP compliance (study considered reliable with restrictions) Male **B6C3F1 mice** 60/group; 15/time point	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %) Oral exposure by gavage Vehicle: water Exposure doses/conc.: 0, 225, 450, 900 mg/kg Exposure: 5 days/week for 7, 14, 28 or 90 days	No mortality. Dose dependent increase in haemolysis, iron deposition in Kupffer cells and oxidative damage. Decreased HCT at 450 and 900 mg/kg bw (- 18 % at 450 mg/kg; - 13 % at 225 mg/kg after 14, 28 and 90 days). Significant depletion of hepatic vitamin E levels. Significantly increased spleen (~ 2-fold) and liver (~ 1.2-fold) weights at 450 and 900 mg/kg bw already after 7 days. Dose and time related increase in Perl's index in Kupffer cells (hepatic deposition of iron; 4 to 14-fold at 450 mg/kg and 14 to 28-fold at 900 mg/kg). LOAEL: 225 mg/kg bw/day.	N/A	Siesky et al. (2002)
Inhalation				
Repeated dose toxicity study: inhalation No TG followed No GLP compliance (study considered reliable with restrictions) Male and female **Fischer 344 rats** 16/sex/group	2-butoxyethanol (CAS: 111-76-2) (purity: 99.4 %) Exposure via inhalation (vapour, whole body) Exposure doses/conc.: 0, 0.10, 0.66, 1.0 mg/L (equivalent to 0, 20, 86, or 245 ppm) Exposure duration: 6 h/day for 9 days in total (5 consecutive days of exposure, followed by 2	No mortality. Significantly lower body weight gain in females at 0.6 mg/L. Haematotoxicity (no quantitative details reported): - significant decrease in RBC counts - significant decrease in Hb and HCT - significant decrease in MCHC at ≥ 1.0 mg/L. - significant increase in MVC, nucleated RBC and reticulocytes at ≥ 1.0 mg/L. Substantial recovery 14 days post exposure in females. Differences remained in males.	N/A	Dodd et al. (1983)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
	days without exposure, then 4 additional consecutive days of exposure).	NOAEL: mg/L/6 h/day (20 ppm).		
OECD TG 414 (Prenatal developmental toxicity study) GLP compliant Deviations from TG: not specified (study considered reliable with restriction) Female **Fischer 344 rats** 36/group	2-butoxyethanol (CAS: 111-76-2) (purity unknown) Exposure via inhalation (vapour) Exposure doses/conc.: 0, 0.12; 0.24; 0.48; 0.97 mg/L; (equivalent to 0, 25, 50, 100, or 200 ppm) Exposure duration: 6 h/day; 10 days (GD 6 – 15) Post exposure period: 6 days	No mortality. Details on maternal toxic effects: Haematuria at ≥ 0.48 mg/L and pale, cold extremities with necrosis of the tail tip at 0.97 mg/L. Weight loss at ≥ 0.48 mg/L. Haematotoxicity: - significant reduction in RBC counts at ≥ 0.48 mg/L (- 10 % at 0.48 mg/L; - 9 % at 0.97 mg/L) - significant increases in MCV and MCH at ≥ 0.48 mg/L (MCV: + 11 % at 0.48 mg/L; + 30 % at 0.97 mg/L; MCH: + 11 % at 0.48 mg/L; + 25 % at 0.97 mg/L). - significant reduction in MCHC at ≥ 0.48 mg/L (- 2 % at 0.48 mg/L; - 5 % at 0.97 mg/L). - significant increase in Hb concentration (+ 14 %) at 0.97 mg/L - significant increase in HCT (+ 20 %) at 0.97 mg/L Increased absolute and relative spleen and relative kidney weight significantly elevated at 0.97 mg/L. NOAEL: 0.24 mg/L (=50 ppm)	0.48 mg/L/6 h/ day - CLP criterion, Cat. 1, study duration 10 days: C ≤ 1.8 mg/L/ 6 h/day - STOT RE Cat. 1	Tyl et al. (1984)
Repeated exposure study No TG followed GLP compliance not	2-butoxyethanol (CAS: 111-76-2) (purity unknown)	No mortality. Haematotoxicity after 4 days of exposure:	0.97 mg/L/6 - 7 h/day -	Mellon Institute of Industrial Research (1952)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
specified (study not assignable) Male and female **rats** Strain and sex not specified 6/group	Exposure via inhalation (vapour) Exposure doses/conc.: 0, 0.97 mg/L (equivalent to 0, 200 ppm) Exposure duration: 6 - 7 h/day for 10 days Post exposure period: 8 days	- Significant decrease in RBC count (- 50%). - Significant decrease in Hb concentration (- 25 %). Haem toxicity after 10 days of exposure: - Significant decrease in RBC count (> 50 %). - Significant decrease in Hb concentration (> 25 %). After post exposure period, values recovered, but RBC counts still subnormal. Normal fragility values.	CLP criterion, Cat. 1, study duration 10 days: C ≤ 1.8 mg/L/ 6 h/day - Animals were exposed for 6 - 7 h instead of only 6 h per day (in total < + 10 h of exposure), however due to the large difference of the respective LOAEL to the guidance value for Cat. 1, a classification as STOT RE Cat. 1 is considered appropriate.	
Repeated exposure study No TG followed No GLP compliance (study considered reliable with restrictions) Male and female **Alderley Park rats** 4/sex/group	2-butoxyethanol (CAS: 111-76-2) (purity unknown) Exposure via inhalation (vapour, whole body) Exposure doses/conc.: 0, 0.1; 0.24; 0.48; 1.2 mg/L (equivalent to 0, 20, 50, 100, 250 ppm) Exposure duration:	Weight loss at 1.2 mg/L, animals lethargic. Organs appeared normal. Haematotoxicity: - increased RBC osmotic fragility at ≥ 0.24 mg/L. - initial haemoglobinuria at 1.2 mg/L - low Hb concentration and MCHC at 1.2 mg/L (no quantitative details reported). Adverse signs seen after 4 days at 1.2 mg/L, exposure to this concentration was not continued.	N/A	Gage (1970)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
(urine, blood, and microscopic examination of lungs, liver, kidneys, spleen, and adrenals only)	6h/day, 5 days/week for 3 weeks	LOAEC: 0.24 mg/L. NOAEC: 0.1 mg/L.		
Short-term repeated dose toxicity study: inhalation No TG followed No GLP compliance (study considered reliable with restrictions) Male and female **Sherman rats** 15/sex/group (blood, liver, lungs and kidneys examined only)	2-butoxyethanol (CAS: 111-76-2) (purity unknown) Exposure via inhalation (vapour, whole body) Exposure doses/conc.: 0, 0.26, 0.52, 0.98, 1.5 (females only), 2.1 mg/L (equivalent to 0, 54, 107, 203, 314 (females only), 432 ppm) Exposure duration: 7h/day, 5 days/ week for 6 weeks	Significantly increased mortality in females at ≥ 1.5 mg/L and males at 2.1 mg/L. No mortality at 0.98 mg/L. No further (temporal) details reported. Haematotoxicity: - haemoglobinuria at ≥ 1.5 mg/L in females and males at 2.1 mg/L (one animal at 0.98 mg/L) - significantly higher erythrocyte fragility at 0.26 mg/L at the end of the study (reversible within 24 h) Significantly higher liver and kidney weights at ≥0.52 mg/L. Pathological effects: - congestion and haemorrhage of lungs - congestion of most of abdominal viscera LOAEL: 0.26 mg/L.	1.5 mg/L/**7 h**/day - CLP crtiteria, Cat. 2, study duration 42 days: 0.4 < C ≤ 2.0 mg/L/**6 h**/day - Animals were exposed for 7 h instead of only 6 h per day (in total + 30 h of exposure), however due to the relatively large difference of the respective LOAEL to the upper and lower guidance values for Cat. 2, a classification as STOT RE Cat. 2 is considered appropriate.	Mellon Institute of Industrial Research (1956) cited in Carpenter et al. (1956)
Sub-chronic toxicity	2-butoxyethanol (CAS: 111-76-2) (purity	Haematotoxicity: Increase in erythrocyte osmotic fragility, statistically	N/A	Anonymous (1970)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
study: inhalation No TG followed No GLP compliance (study considered reliable with restrictions) Male **Carworth E rats** 32/sex/dose	unknown) Exposure via inhalation (vapour, whole body) Exposure dose/conc.: 0.24 mg/L (50 ppm) Exposure duration: 7h/day, 5 days/ week for 90 days	significant at study end (+ 30 %) Significantly increased relative (but not absolute) kidney weight (+ 6.2 %). No further details reported. LOAEL: 0.24 mg/L.		
Similar to OECD TG 413 (Subchronic Inhalation Toxicity: 90-day Study) GLP not specified but assumed Deviations from TG: some rats killed after 42 days. Gross and histopathological examinations only in rats from controls and highest dosage group. (study considered reliable with restriction) Male and female **Fischer 344 rats** 16/sex/group	2-butoxyethanol (CAS: 111-76-2) (purity > 99.4 %) Exposure via inhalation (vapour) Exposure doses/conc.: 0, 0.02, 0.12 and 0.37 mg/L (equivalent to 0, 5, 25, 77 ppm) Exposure duration: 6 hours/day, 5 days/week for 42 or 90 days Post exposure period: not specified	No mortality but transient decrease in body weight gain at 0.37 mg/L. Significant haematological effects at 0.37 mg/L (with effects greater after 6 weeks than at 13 weeks): - decrease in RBC count - decrease in Hb concentration - decrease in HCT - increase in MCH No quantitative details reported. NOAEL: 0.121 mg/L/6 h/day (= 24.6 ppm)	N/A	Bushy Run Research Center (1981b)
Similar to OECD TG 413 (Subchronic	2-butoxyethanol (CAS:	Significant lower body weight gain in females at 0.37 mg/L.	0.37 mg/L/6 h/day	Dodd et al. (1983)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
Inhalation Toxicity: 90-day Study) GLP compliance not specified Deviations from TG: not specified (study considered reliable with restrictions) Male and female **Fischer 344 rats** 16/sex/group	111-76-2) (purity: 99.4 %) Exposure via inhalation (vapour, whole body) Exposure doses/conc.: 0, 0.02, 0.12, 0.37 mg/L (equivalent to 0, 5, 25 or 77 ppm) Exposure duration: 6 h/day, 5 days/week for 90 days	Haematotoxicity: - 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. Effects more pronounced in females than males. At the end of the 90-day study, haematologic effects either lessened or returned to control ranges; no longer statistically significant. NOAEL: 0.12 mg/L/6 h/day (25 ppm) for females; 0.37 mg/L/6 h/day (77 ppm) for males.	- CLP crtiteria, Cat. 2, study duration 90 days: $0.2 < C \leq 1.0$ mg/L/6 h/day - STOT RE Cat. 2	
OECD TG 453 (Combined chronic toxicity/ carcinogenicity studies) GLP compliant No deviations from TG. (study considered reliable without restrictions) Male and female **Fischer 344 rats** 50/sex/group	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %) Exposure by inhalation (vapour) Exposure doses/conc.: 0, 0.15, 0.3, 0.6 mg/L (equivalent to 0, 31, 62.5, 125 ppm) Exposure duration: 6 h/day plus chamber equilibration time (12 min), 5 days/week, for 3, 6, 12 and 24 months	Survival of treated rats similar to the controls. Decreased body weight in females at 0.6 mg/L. 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 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 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 (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	0.15 mg/L/6 h/day (based on reduction (> 10 %) in Hb concentration) - CLP crtiteria, Cat. 2, study duration **6 months**: $0.1 < C \leq 0.5$ mg/L/6 h/day - STOT RE Cat. 2	NTP (2000)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
		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 males (at 0.6 mg/L) and females (at 0.3 mg/L) 15% to 35% decreases in the myeloid/erythroid (M/E) ratio in bone marrow at 0.6 mg/L in both sexes. Females exposed to 0.3 mg/L generally had reduced M/E ratios of 10% to 30%. Cytological, morphologic alterations and megakaryocytes present in all exposure groups. Histopathologic effects (after 2 years): - significantly increased hyaline degeneration of the olfactory epithelium in males at all concentrations - Kupffer cell pigmentation in liver in both sexes at ≥ 0.3 mg/L (linear dose-response) - spleen fibrosis in males at ≥ 0.3 mg/L	---------- 0.6 mg/L/6 h/day (based on reduction (> 10 %) in RBC count and Hb concentration) - CLP crtiteria, Cat. 2, study duration **365 days**: 0.05 < C ≤ 0.25 mg/L/6 h/day - STOT RE No classification ---------- 0.3 mg/L/6 h/day (based on occurrence of spleen fibrosis at this concentration) - CLP crtiteria, Cat. 2, study duration **730 days**:	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
			0.03 < C ≤ 0.13 mg/L/6 h/day - STOT RE No classification	
Repeated dose toxicity study No TG followed No GLP compliance Male and female **Fischer 344/N rats** 10/sex/group	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %) Exposure via inhalation (vapour, whole body) Exposure doses/conc.: 0, 0.15, 0.3, 0.6, 1.2, 2.4 mg/L (equivalent to 0, 31, 62.5, 125, 250, 500 ppm) Exposure duration: 6 h/day, 5 days/week for 13 weeks	Mortality: 5/10 females killed moribund at 2.4 mg/L. Haematotoxicity (at 2.4 mg/L): - macrocytic, normochromic, and regenerative anaemia (no details reported) anemia - 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	N/A (due to lack of data on effects of concentrations < 2.4 mg/L)	Nyska et al. (1999)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
		- inflammation, necrosis, ulceration, and hyperplasia of the forestomach - centrilobular degeneration of the liver - haemoglobinuric nephrosis No details on effects at < 2.4 mg/L reported.		
Repeated dose toxicity study No TG followed No GLP compliance Female **Fischer 344/N rats** 10/group	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %) Exposure via inhalation (vapour) Exposure doses/conc.: 0, 0.15, 0.3, 0.6, 1.2, 2.4 mg/L (equivalent to 0, 31, 62.5, 125, 250, 500 ppm) Exposure duration: 6 h/d, 5 d/week for 13 weeks	5/10 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	0.3 mg/L/6 h/day (based on reduction (> 10 %) in RBC count at this concentration) - CLP criteria, Cat. 2, study duration 90 days: 0.2 < C ≤ 1.0 mg/L/6 h/day - STOT RE Cat. 2	Long et al. (2000)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
		- degenerative changes in ameloblast layers No thrombosis or degeneration at 1.2 mg/L.		
OECD TG 413 (Subchronic inhalation toxicity: 90-day study) GLP compliant Deviations from TG: no clinical chemistry, urine analysis and ophthalmology. (study considered reliable without restrictions) Male and female **Fischer 344 rats** 10/sex/group	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %) Exposure by inhalation (vapour, whole body) Exposure doses/conc.: 0, 0.15, 0.3, 0.6, 1.2, 2.4 mg/L (equivalent to 0, 31, 62.5, 125, 250, 500 ppm) Exposure duration: 6 h/day plus chamber equilibration time (12 min), 5 days/week, 14 weeks	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	0.6 mg/L/6 h/day (based on reduction (> 10 %) in RBC count and Hb concentration) - CLP crtiteria, Cat. 2, study duration 98 days: 0.2 < C ≤ 1.0 mg/L/6 h/day - STOT RE Cat. 2	NTP (2000)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
		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 Kupffer 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) LOAEL: 0.15 mg/L for females and 0.6 mg/L for males. NOAEL: 0.3 mg/L for males.		
Sub-chronic toxicity study: inhalation No TG followed No GLP compliance (study considered reliable with restrictions) Male **mice** Strain: not specified No. of animals not	2-butoxyethanol (CAS: 111-76-2) (purity unknown) Exposure via inhalation (vapour, whole body) Exposure doses/conc.: 0, 0.48, 0.97, 1.93, mg/L (equivalent to 0, 100, 200, 400 ppm) Exposure duration:	No mortality. Haematotoxicity: - haematuria at all concentrations (linear dose-response; recovered after 3 exposures) - significant increase in erythrocyte fragility (recovered after 17 h prost exposure)	N/A	Mellon Institute of Industrial Research (1956) cited in Carpenter et al. (1956)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
specified	7h/day, 5 days/ week for 90 days Post exposure period: 42 days	Significantly increased liver weights at 1.93 mg/L (recovered within 42 days post exposure). LOAEL: 0.48 mg/L.		
OECD TG 413 (Subchronic inhalation toxicity: 90-day study) GLP compliant Deviations from TG: no clinical chemistry, urine analysis and ophthalmology. (study considered reliable without restrictions) Male and female **B6C3F1 mice** 10/sex/group	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %) Exposure by inhalation (vapour, whole body) Exposure doses/conc.: 0, 0.15, 0.3, 0.6, 1.2, 2.4 mg/L (equivalent to 0, 31, 62.5, 125, 250, 500 ppm) Exposure duration: 6 h/day plus chamber equilibration time (12 min), 5 days/week, 14 weeks	Mortality: 2.4 mg/L: 2 male and two female killed moribund during the first 2 weeks. Animals showed abnormal breathing, red urine stains and lethargy. Significant lower body weight and body weight gains at ≥ 0.6 mg/L. 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 Females more sensitive than males. Increase in relative liver weights at 1.2 mg/L in males and 2.4 mg/L in females.	1.2 mg/L/6 h/day - CLP crtiteria, Cat. 2, study duration 98 days: 0.2 < C ≤ 1.0 mg/L/6 h/day - STOT RE No classification	NTP (2000)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
		Histopathologic effects: - lymphoid atrophy of the spleen, thymus, and mesenteric and mandibular lymph nodes occurred 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 in male mice 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 Kupffer 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 LOAEL: 0.15 mg/L for females and 0.6 mg/L for males. NOAEL: 0.3 mg/L for males.		
OECD TG 453 (Combined chronic toxicity/ carcinogenicity studies) GLP compliant No deviations from TG. (study considered reliable without	2-butoxyethanol (CAS: 111-76-2) (purity: > 99 %) Exposure by inhalation (vapour) Exposure doses/conc.: 0, 0.3, 0.6, 1.2 mg/L (equivalent to 0, 62.5, 125, 250 ppm)	Significantly higher mortality of male mice at ≥ 0.6 mg/L (no (temporal) 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	1.2 mg/L/6 h/day (based on reduction (> 10 %) in Hb concentration) - CLP crtiteria, Cat. 2, study duration **6 months**: 0.1 < C ≤ 0.5 mg/L/6 h/day	NTP (2000)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
restrictions) Male and female **B6C3F1 mice** 50/group	Exposure duration: 6 h/day plus chamber equilibration time (12 min), 5 days/week for 3, 6, 12 and 24 months	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 - only changes in MCV in females after 12 months at 1.2 mg/L - no changes in MCH and MCHC - 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: - significant increase of focal epithelial hyperplasia in both sexes at all concentrations, in females usually associated with ulceration - significantly increased no. of haemangiosarcoma in liver of male mice at 1.2 mg/L, sometime also in bone marrow and heart or bone marrow and spleen - haemosiderin pigmentation in Kupffer cells in males exposed ≥ 0.6 mg/L and females at ≥ 0.3 mg/L - increased haematopoietic cell proliferation in spleen at ≥ 0.6 mg/L in males and 1.2 mg/L in females	- STOT RE No classification ---------- 1.2 mg/L/6 h/day (based on reduction (> 10 %) in RBC count and Hb concentration) - CLP crtiteria, Cat. 2, study duration **365 days**: 0.05 < C ≤ 0.25 mg/L/6 h/day - STOT RE No classification ---------- 0.6 mg/L/6 h/day (based on histopathological findings)	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
		- increased haemosiderin pigmentation in spleen at ≥ 0.3 mg/L in males and ≥ 0.6 mg/L in females - increased incidences of hyperplasia in males at ≥ 0.6 mg/L - hyaline degeneration in olfactory epithelium and respiratory epithelium in females at all concentrations - glomerulosclerosis and hydronephrosis in males at ≥ 0.6 mg/L LOAEL: 0.3 mg/L for females and 0.6 mg/L for males.	- CLP crtiteria, Cat. 2, study duration **730 days**: 0.03 < C ≤ 0.13 mg/L/6 h/day - STOT RE No classification	
OECD TG 414 (Prenatal developmental toxicity study) GLP compliant Deviations from TG: not specified (study considered reliable with restriction) Female **New Zealand White rabbits** 24/group	2-butoxyethanol (CAS: 111-76-2) (purity unknown) Exposure via inhalation (vapour) Exposure doses/conc.: 0, 0.12; 0.24; 0.48; 0.97 mg/L; (equivalent to 0, 25, 50, 100, 200 ppm) Exposure duration: 6 h/day; 13 days (GD 6 – 18)	Details on maternal toxic effects: Mortality at 0.97 mg/L (4/20) 3 days after exposure start. Significantly lower body weight and body weight gain (linear dose-response) at 0.97 mg/L (8 %). Haematotoxicity (no quantitative details reported): - no apparent haematological effects - significant increases in Hb content and HCT at 0.48 mg/L but increase was not significant at 0.97 mg/L LOAEC: 0.97 mg/L. NOAEC: 0.48 mg/L.	N/A	Tyl et al. (1984)
Short-term repeated dose toxicity study: inhalation	2-butoxyethanol (CAS: 111-76-2) (purity	2/10 male guinea pigs died at 2.4 mg/L after 12 days and 1/10 at 1.8 mg/L after 7 days.	1.8 mg/L/**7 h**/day -	Mellon Institute of Industrial Research

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
No TG followed No GLP compliance (study considered reliable with restrictions) Male and female **guinea pigs** Strain: not specified Test 1: 10males/group Test 2: 6/sex/group	unknown) Exposure via inhalation (vapour, whole body) Exposure doses/conc.: Test1 and 2: 0, 1.8, 2.4 mg/L (equivalent to 0, 375 and 500 ppm) Exposure duration: 7h/day, 7 days/week for 30 days Post exposure period: not specified	Significantly lower body weight in females at 1.8 mg/L (not at higher concentration). Haematotoxicity (at ≥ 1.8 mg/L). No haematuria. Lung haemorrhage and lung congestion at 1.8 mg/L. Significantly higher kidney weights in females at ≥ 1.8 mg/L (<10%). LOAEL: 1.8 mg/L.	CLP crtiteria, Cat. 2, study duration 30 days: 0.6 < C ≤ 3.0 mg/L/**6 h**/day - Animals were exposed for 7 h instead of only 6 h per day (in total + 30 h of exposure), however due to the large difference of the respective LOAEL to the upper and lower guidance values for Cat. 2, a classification as STOT RE Cat. 2 is considered appropriate.	(1956) cited in Carpenter et al. (1956)
Short-term repeated dose toxicity study: inhalation No TG followed No GLP compliance (study considered reliable with restrictions) Male and female **Basenji or Wire-haired terrier dogs**	2-butoxyethanol (CAS: 111-76-2) (purity unknown) Exposure via inhalation (vapour) Exposure doses/conc.: Test1 and 2: 0, 0.48, 0.97, 1.86 mg/L (equivalent to 0, 100, 200 and 385 ppm)	100 % mortality at 1.86 mg/L after 28 days and 8 days of exposure, respectively (previous symptoms: weakness, apathy, anorexia, weight loss) At 0.97 mg/L slight evidence of toxicity after 31 days of exposure. Haematotoxicity: - significantly increase in erythrocyte fragility in both sexes after 7 days of exposure to 1.86 mg/L; followed by decrease until end of study - Slight but significant increase in erythrocyte fragility in	1.86 mg/L/**7 h**/day - CLP criterion, Cat. 1, study duration **8 days**: C ≤ 2.0 mg/L/ **6 h**/day CLP criteria, Cat. 2, study duration **8 days**: 2.0 < C ≤ 10 mg/L/ **6 h**/day	Mellon Institute of Industrial Research (1956) cited in Carpenter et al. (1956)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
1/sex/group	Exposure duration: 7h/day, 7 days/ 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.	both sexes at 0.97 mg/L - Slight but significant decrease of Hb concentration throughout the study at 0.97 mg/L - Slight but significant decrease in HCT at 0.48 mg/L - Significantly elevated plasma fibrinogen concentrations at 1.86 mg/L Histopathology: - congestion of liver and lungs at 1.86 mg/L - congestion of kidneys only in females LOAEL: 0.48 mg/L.	- Animals were exposed for 7 h instead of 6 h per day (in total + 8 h of exposure); due to the relatively small difference of the respective LOAEL to the guidance value of STOT RE Cat. 1, a classification as STOT RE Cat. 2 is considered appropriate. ------ 1.86 mg/L/**7 h**/day - CLP crtiteria, Cat. 2, study duration **28 days**: $0.6 < C \leq 3.0$ mg/L/**6 h**/day - Animals were exposed for 7 h instead of only 6 h per day (in total + 28 h of exposure), however due to the large	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
			difference of the respective LOAEL to the upper and lower guidance values for Cat. 2, a classification as STOT RE Cat. 2 is considered appropriate.	
Chronic toxicity study: inhalation No TG followed No GLP compliance (study considered reliable with restrictions) Female **Rhesus monkeys** 1/group	2-butoxyethanol (CAS: 111-76-2) (purity unknown) Exposure via inhalation (vapour) Exposure doses/conc.: Test1: 0, 0.48, 0.97 mg/L (equivalent to 0, 100, 200 ppm) Test 2: 0.48 mg/L (100 ppm) for 10 days, then 0.97 mg/L (200 ppm) Exposure duration: Test 1: 7h/day, 5 days/ week for 90 days Test 2: 7h/day 0.48 mg/L (100	In group 2, one animal died of causes unrelated to treatment. Haematotoxicity (no quantitative details reported): - test 1: No changes in erythrocyte fragility during 0.48 mg/L exposure, but increased at 0.97 mg/L (recovered until end of study) - test 2: increase in erythrocyte fragility at ≥ 0.48 mg/L (approx. 21 % after the 18th exposure; in females 35 % after 7 exposures). Recovery by the end of the exposure period LOAEL: 0.48 mg/L.	N/A	Mellon Institute of Industrial Research (1956) cited in Carpenter et al. (1956)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
	ppm) for 10 days, then dose was increased to 0.97 mg/L (200 ppm) for 80 days			
Dermal				
OECD TG 411 (Subchronic dermal toxicity: 90-day study) GLP compliant Deviations from TG: not specified (study considered reliable without restriction) Male and female **New Zealand White rabbits** 10/sex/group	2-butoxyethanol (CAS: 111-76-2) (purity unknown) Exposure via occlusive dermal application Vehicle: distilled water (1mL/day) Exposure doses/conc.: 0, 2.8, 14.3, 42.8 % aqueous solutions (equivalent to 0, 10, 50 and 150 mg/kg bw, respectively) Exposure duration: 6 hours/day, 5 days/week for 13 weeks	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 No (histo)pathological changes of organs. No changes in organ weight. NOAEL: 150 mg/kg/day.	N/A	Wil Research Laboratories (1983)
Short-term repeated dose dermal toxicity study No TG followed No GLP compliance assumed (study not assignable)	2-butoxyethanol (CAS: 111-76-2) (purity unknown) Exposure via occlusive dermal application Vehicle: assumed to be water	No mortality. Decreased body weight gain in female rabbits treated 100 % 2-butoxyethanol throughout the study. Haematotoxicity: - haemoglobin in urine 2/4 males on day 2 -5 at 100 %, and in 4/5 females at a concentration of 100 % (undiluted) and 5/5 at 50 % up to day 9	900 mg/kg bw/day - CLP crtiteria, Cat. 2, study duration 9 days: $200 < C \leq 6000$ mg/kg bw/day	Bushy Run Research Center (1989)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
Male and female **New Zealand White rabbits** 5/sex/group	Exposure doses/conc.: 100, 50, 25, 5, 0 % (1 mL/kg of mixture corresponding to 900, 450, 225, 45 mg/kg bw) Exposure duration: 6 hr/day for 9 days (dosed for 5 days, no dosing for 2 days and then dosed for further 4 days) Post exposure period: 14 days	- significantly decreased RBC counts, Hb concentration and MCHC and increased MCH on day 9 in females at a concentration of 100 % (undiluted) - decreased HCT and increased MCV (recovered after post exposure period) No changes in any organ weight but dose related patchy colour change of the kidneys of 3 females at a concentration of 100 % (undiluted).	- STOT RE Cat. 2	
Other routes				
Haematotoxicity study, *in vivo* No TG followed No GLP compliance (study considered reliable with restrictions) Male **Wistar rats** 5/group	2-butoxyethanol (CAS: 111-76-2) (purity unknown) Subcutaneous exposure Vehicle: saline Exposure doses/conc.: 0, 0.25, 0.5, 0.75, 1.25 mM/kg bw/day (equivalent to 0, 30, 60, 88, 148 mg/kg bw/day) Exposure duration: daily, 5 days/week for 0, 4, 11 and 29 days	Haematotoxicity: - significantly decreased RBC counts throughout the study at ≥ 60 mg/kg bw/day; at day 11 significantly reduced at ≥ 30 mg/kg bw/day - significantly decreased Hb concentrations after 4 (and 11) days at ≥ 60 mg/kg bw/day, recovered until day 18 (max. 13 % at 60 mg/kg bw/day; max. 53 % at 148 mg/kg bw/day) - significantly increased MCH - significantly increased MCV throughout the study, significant after 4 weeks at ≥ 60 mg/kg bw/day; significant on days 11 and 18 at ≥ 30 mg/kg bw/day	148 mg/kg bw/day - N/A	Starek et al. (2008)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results, overall LOAEL and (if applicable) NOAEL of the study results**	**LOAEL** specifically related to criteria on haemolytic anaemia - **CLP criteria met** standard guidance values adjusted for varying study durations, using dose/exposure time extrapolations similar to Haber's rule - **Classification/ category**	**Reference**
Haematotoxicity study, in vivo No TG followed No GLP compliance (study considered reliable with restrictions) Male **Wistar rats** 5/group	2-butoxyethanol (CAS: 111-76-2) (purity unknown) Subcutaneous exposure Vehicle: saline Exposure doses/conc.: 0.75, 1.25 mM/ kg bw/day (equivalent to 88, 148 mg/kg bw/day) Exposure duration: daily, 5 days/week for 4 weeks Haematological analyses were performed on day 0, 4, 11, 18, and 29.	Haematotoxicity: - significant decrease in RBC counts at ≥ 88 mg/kg bw/day throughout the study duration (max. 30 % and 40 % at 88 and 148 mg/kg bw/day , respectively) - significant decrease in Hb concentration on the days 4, 11 and 29 at 88 mg/kg bw/day and on the days 4, 18 and 29 at 148 mg/kg bw/day (max. 30 % and 75 % at 88 and 148 mg/kg bw/day , respectively after 4 days) - significantly increased MCV throughout the study duration at ≥ 88 mg/kg bw/day - significantly increased reticulocytes on the days 4 and 11 at 88 mg/kg bw/day and throughout the study duration at 148 mg/kg bw/day Haemoglobinuria on the first day of exposure. Reduced lymphocyte counts at ≥ 88 mg/kg bw/day after 11 days until end of the study.	88 mg/kg bw/day - N/A	Starek-Swiechowicz et al. (2015)

Table 22: Summary table of human data on STOT RE

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
Study type: Case report, suicide attempt	Glass cleaner containing 22 % 2-butoxyethanol	Ingestion of 360 - 480 mL glass cleaner (max. 95 g of 2-butoxy-ethanol: ~ 1.25 g/kg bw) Nine days following the initial discharge, the patient was again admitted after ingestion of 480 mL of the same cleaner. Number of subjects exposed: 1 Sex: male Age: 18 years old	First incident: - Hepatic biochemical abnormalities (Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT) and hepatic bilirubin). - No haematological and renal abnormalities No metabolic or hepatic, haematological or renal abnormalities at the second hospitalisation.	Gualtieri et al. (1995) and Gualtieri et al. (2003)

9.6.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Animal studies

There are various *in vivo* studies available, which provide sufficient data for CLP classification regarding STOT RE and which are considered relevant and reliable (see Table 21), including studies which were performed according to validated OECD TGs and/or GLP, as well as studies which were performed especially to assess the haematotoxic potential of 2-butoxyethanol under various conditions.

Most of the relevant and reliable studies described in Table 21 indicate that 2-butoxyethanol causes severe haemolytic anaemia in various mammals, such as rats, mice, rabbits, guinea pigs, dogs and monkeys, independent of the route of exposure. Key effects include in all species drastic reductions in RBC counts, Hb concentrations (both usually > 10 % and up to > 50 %) and HCT. Further effects are significant increases in erythrocyte fragility, MCV and MCH, indicating erythrocyte deformation and swelling (macrocytosis) e.g. due to increased (secondary) reticulocytosis. In most cases, an increase in haemosiderin (iron) deposition in liver (Kupffer cells), spleen and/or kidneys as indicators for severe chronic haemolysis, as well as haemoglobinuria could be observed. An increase in extramedullary haematopoiesis in spleen and/or liver was also noted in several studies. The multiplicity of affected organs hereby reflects the severity of haemolysis: ‘when the extent of haemolysis is extensive haemosiderin may be deposited in the liver, spleen, kidney, bone marrow and other organs’ (Muller et al., 2006). A significant increase in haemosiderin accumulation in organs is not fully reversible and is associated with several adverse effects in the respective organs, such as fibrosis and cell death (Muller et al., 2006). Accordingly, in most *in vivo* studies listed in Table 21 (severe) organ dysfunction as a result of multifocal lesions, such as systemic and microvascular thrombosis, cell degeneration, fibrosis and/or necrosis due to infarction especially in eyes and tail, but also in liver, spleen and other organs, could be detected. Direct effects of 2-butoxyethanol as a cause of those lesions can also not be ruled out. Furthermore, as an adaptive response to the systemic haemolysis an increase in erythropoiesis in bone marrow and in the number of reticulocytes in the

blood were noted. However, an abnormal morphology of newly formed RBCs was observed in some studies, suggesting a dysfunction of those newly produced cells.

Key effects occurred at concentrations > approx. 10 mg/kg bw/day during/after oral administration in mice and rats (no other species tested), and at ≥ 0.3 mg/L/6 h/day during/after inhalative exposure (vapour) in rats. In mice, key effects were observed during/after inhalative exposure (vapour) at 1.2 mg/L/6 h/day, and in guinea pigs and dogs, respectively, at 0.6 mg/L/7 h/day. Dermal exposure resulted in key effects at a concentration of 90 mg/kg bw/day in rabbits (no other species tested). The reported effective concentrations are extrapolated to a study duration of 90 days using dose/exposure time extrapolations similar to Haber's rule (for details see Table 21 and Guidance on the Application of the CLP Criteria, version 4.1), as guidance values refer to effects seen in a standard 90-day toxicity study conducted in rats.

Results of some studies, as well as predictions generated by the PBPK model indicated that humans are less sensitive to the haemolytic effects of 2-butoxyethanol compared to other species such as rats and mice. Since mechanistic *in vitro* studies demonstrated that the metabolite BAA is likely involved in and may be the main responsible agent for the haematotoxicity caused by exposure to 2-butoxyethanol in most mammals, the proposed differences between species were suggested to be due to the slower metabolic rate and the lower percentages of 2-butoxyethanol being converted to BAA in humans e.g. versus rats, as well as the lower susceptibility of human erythrocytes to BAA effects *in vitro* compared to rat erythrocytes. In contrast, 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 BAA. Hence, the detailed mechanisms of action of 2-butoxyethanol and its metabolites by which severe haemolysis can be caused are not yet fully unravelled (Section 8). Critical analysis of the proposed high interspecies difference is thus essential, also because in almost all of the studies involving human volunteers, the number of tested individuals was low and subjects were exposed to 2-butoxyethanol acutely, only once. Consequences of a repeated or chronic exposure to this substance were never assessed in humans. In some case reports of suicide attempts, moreover, where humans consumed single oral doses of 2-butoxyethanol, e.g. in cleaning formulations, some haemolytic effects have been described in addition to more debilitating effects (see Sections 8 and 9.1). Another factor that needs to be taken into account when assessing the health hazard potential of 2-butoxyethanol is the high interindividual variation in permeation, absorption and elimination of 2-butoxyethanol detected in studies performed on human volunteers. Thus, the possibility exists that some humans, especially certain human subpopulations, including the elderly and those predisposed to haemolytic disorders, might be at increased risk from 2-butoxyethanol exposure, although some *in vitro* studies suggest the contrary (Udden, 1994; Udden, 2002).

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

A number of further *in vivo* studies examining the haemolytic effects of a repeated oral, inhalative and dermal exposure to 2-butoxyethanol for various species are available, but many of these studies were not assignable and/or do not provide sufficient information for interpretation and indisputable classification according to CLP regulations (Anonymous, 1970; Bushy Run Research Center, 1981a; Carpenter et al., 1956; Dodd et al., 1983; Eastman Kodak, 1982; Gage, 1970; Green et al., 2002; Koshkaryev et al., 2003; Myler et al., 2004a; Myler et al., 2004b; Nagano et al., 1984; Nyska et al., 1999; Siesky et al., 2002; Tyl et al., 1984; Wier et al., 1987; Wil Research Laboratories, 1983).

9.6.2 Comparison with the CLP criteria

According to CLP regulation Annex I, section 3.9.2, target organ toxicity (repeated exposure) means specific, target organ toxicity arising from a repeated exposure to a substance or mixture. All significant health effects that can impair function, reversible and irreversible, immediate and/or delayed are included. Other specific toxic effects that are specifically addressed in Sections 9.1, 9.2 and 9.3 are not included.

Classification for target organ toxicity (repeated exposure) identifies the substance or mixture as being a specific target organ toxicant and, as such, it may present a potential for adverse health effects in people who are exposed to it. Assessment shall take into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs. Effects that are considered to support classification for specific target organ toxicity following repeated exposure are among others morbidity or death resulting from repeated or long-term exposure, any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters, significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination, multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity, morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction or evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration.

Specific target organ toxicity can occur by any route that is relevant for humans, i.e. principally oral, dermal or inhalation. The information required to evaluate specific target organ toxicity comes either from repeated exposure in humans, such as exposure at home, in the workplace or environmentally, or from studies conducted in experimental animals. The standard animal studies in rats or mice that provide this information are 28 day, 90 day or lifetime studies (up to 2 years) that include haematological, clinicochemical and detailed macroscopic and microscopic examination to enable the toxic effects on target tissues/organs to be identified. Data from repeat dose studies performed in other species shall also be used, if available. Other long-term exposure studies such as studies on carcinogenicity, neurotoxicity or reproductive toxicity, may also provide evidence of specific target organ toxicity that could be used in the assessment of classification.

Substances are classified as specific target organ toxicants following repeated exposure by the use of expert judgement, on the basis of the weight of all evidence available, including the use of recommended guidance values which take into account the duration of exposure and the dose/concentration which produced the effect(s), and are placed in one of two categories, depending upon the nature and severity of the effect(s) observed.

Substances are classified as STOT RE 1, if they have produced significant toxicity in humans or if, on the basis of evidence from studies in experimental animals, the substance can be presumed to have the potential to produce significant toxicity in humans following repeated exposure (CLP regulation, Annex I, section 3.9.2.1).

Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of:

- reliable and good quality evidence from human cases or epidemiological studies; or
- observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations.

Guidance dose/concentration values are to be used as part of a weight-of- evidence evaluation. The guidance values refer to effects seen in a standard 90-day toxicity study conducted in rats. They can be used as a basis to extrapolate equivalent guidance values for toxicity studies of greater or lesser duration, using dose/exposure time extrapolation similar to Haber's rule for inhalation, which states

essentially that the effective dose is directly proportional to the exposure concentration and the duration of exposure (e.g. for a 28-day study the guidance values below are increased by a factor of three).

Guidance values for STOT RE 1 (CLP regulation, Annex I, Table 3.9.2):

Oral: C ≤ 10 mg/kg bw/day

Dermal: C ≤ 20 mg/kg bw/day

Inhalation (vapour): C ≤ 0.2 mg/L/6h/day

Substances are classified as STOT RE 2 if, on the basis of evidence from studies in experimental animals, it can be presumed that it has the potential to be harmful to human health following repeated exposure.

Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations (CLP regulation, Annex I, Section 3.9.2.1).

Guidance values for STOT RE 2 (CLP regulation, Annex I, Table 3.9.3):

Oral: 10 < C ≤ 100 mg/kg bw/day

Dermal: 20 < C ≤ 200 mg/kg bw/day

Inhalation (vapour): 0.2 < C ≤ 1.0 mg/L/6h/day

The presented guidance values again refer to effects seen in a standard 90-day toxicity study conducted in rats and need to be extrapolated to equivalent guidance values for toxicity studies of greater or lesser duration, using dose/exposure time extrapolation similar to Haber's rule for inhalation (see above).

The guidance values are in general intended only for guidance purposes, e.g. in a weight of evidence approach, to assist with decisions about classification. They are not intended as strict demarcation values.

Regarding the adverse key effects of exposure to 2-butoxyethanol demonstrated in most of the relevant and reliable studies listed in Table 21, a classification of 2-butoxyethanol as STOT RE 2 is warranted irrespectively of the route of exposure. Some single studies, however, could not demonstrate any relevant effects for STOT RE classification. Single further studies, on the other hand, showed key effects of an exposure to 2-butoxyethanol, which warrant a classification as STOT RE 1. Regarding those diverse results, it is noteworthy that the haemolytic effects seem to be in part reversible and that humans seem to be less sensitive to the haemolytic effects caused by this substance than e.g. rats and mice, although interindividual variation is very high in humans. Hence, a classification of 2-butoxyethanol as STOT RE 2 is considered justified.

Specific concentration limits (SCLs) for STOT RE classification only need to be set, if the respective substance induces target organ toxicity at a dose level or concentration clearly (more than one magnitude) below the guidance values for Category 1 according to CLP regulation, Annex I, Table 3.9.2. It is not appropriate to determine SCLs for substances classified as Category 2 since ingredients with higher potency (i.e. lower effect doses than the guidance values of Category 2) will be classified in Category 1 and substances with respective higher effective doses will generally not be classified.

9.6.3 Conclusion on classification and labelling for STOT RE

According to CLP 2-butoxyethanol has to be classified as:

STOT RE 2 and labelled with hazard statement H373: "May cause haemolytic damage through prolonged or repeated (oral, inhalative and dermal) exposure.", with the pictogram "GHS08: Health Hazard", and with the signal word "Warning".

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).

<u>Oral exposure</u>

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 ≤ 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 ($0.2 < C \leq 1.0$ 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: $0.6 < C \leq 3.0$ 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 ($0.2 < C \leq 1.0$ 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 7^{th} and 18^{th} 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

GVs for STOT RE 2 referring to effects seen in a standard 28-d toxicity study conducted in rats:
- Oral: $30 < C \leq 300$ mg/kg bw/d
- Dermal: $60 < C \leq 600$ mg/kg bw/d
- Inhalation (vapour): $0.6 < C \leq 3.0$ mg/L/6h/d

Oral route

In a repeat dose toxicity study (Kenyon *et al*., 2015, non-GLP, non-guideline), male rats were given 2-buthoxyethanol by gavage at doses of 10, 100, 250 and 450 mg/kg bw/d for 28 days; post exposure period 7 days.

Effects observed:
- no mortality;
- significant lower body weight gain or body weight loss during study day 1 - 15 at ≥ 250 mg/kg bw/d (recovered by day 29).

Haematotoxicity:
- haemoglobinuria at ≥ 00 mg/kg bw/d (3/6 at lowest dose, 100% at higher doses); recovery within 1 week;
- significant decrease in RBC count at ≥ 100 mg/kg bw/d after 2 and 8 days of exposure (-12% at 100 and -44% at 450 mg/kg bw/d, respectively); recovered slowly (still significant at ≥ 250 mg/kg bw/d after 35 days);
- significant decrease in Hb concentration at ≥ 100 mg/kg bw/d after 2 and 8 days of exposure:
    - after 2 days: -12% at 100 mg/kg bw/d, -33% at 250 mg/kg bw/d, and -24% at 450 mg/kg bw/d, respectively;
    - after 8 days: -12% at 100 mg/kg bw/d, -14% at 250 mg/kg bw/d, and -45% at 450 mg/kg bw/d, respectively; recovered until day 35;
- significant decrease in HCT at ≥ 100 mg/kg bw/d after 2 and 8 days of exposure (-11% at 100 and -40% at 450 mg/kg bw/d); recovered until day 35;
- significant increase in MCV at ≥ 250 mg/kg bw/d until end of study (day 35)

Conclusion: Since no detailed data on Hb and RBC count are provided, the results cannot be compared with the classification criteria. High decrease of Hb of -33% and -14% after 2 and 8 days of exposure, respectively, at 250 mg/kg bw/d, and of -24% and -45% after 2 and 8 days of exposure at 450 mg/kg bw/d, shows a high haemolytic potential of 2-buthoxyethanol in rats. However, these effects, particularly, after 2 days, are rather due to acute toxicity of the substance, and no to repeated dose toxicity, so it is questionable whether they are sufficient for classification for repeated dose toxicity. The Hb values after a recovery period of 7 did not differ from control values even at 450 mg/kg bw/d.

In a repeat dose toxicity study (Eastman Kodak, 1982; OECD TG 407, non-GLP) male rats were given 2-buthoxyethanol by gavage at doses of 222, 443, and 885 mg/kg bw/d for 5 d/week, for 6 weeks.

Effects observed:
- 2 spontaneous deaths in the high dose group only;
- blood in urine at ≥ 443 mg/kg bw/d throughout the study, and in 1 rat at 222 mg/kg bw/d after 3 weeks;
- lethargy, unkempt hair coats, piloerection, rales, slight weakness and inactivity at ≥ 443 mg/kg bw/d;
- significant body weight reduction at 885 mg/kg bw/d after 13 days;
- increased spleen weight at ≥ 443 mg/kg bw/d, spleen enlarged and dark;
- splenic congestion and extramedullary haematopoiesis in spleen at all doses;

- increased liver weight at ≥ 222 mg/kg bw/d;
- liver lesions at ≥ 443 mg/kg bw/d:
  - heptocytomegally (high dose only)
  - anisokaryosis (low and mid dose);
  - and haemosiderin deposition (high and mid dose);
- stomach hyperkeratosis at all doses;
- renal effects:
  - proteinaceous casts and haemosiderin in the proximal convoluted tubules at ≥ 443 mg/kg bw/d.

Haematotoxicity:
- decrease in Hb concentration, total RBC count;
- increase in MCHC at all doses (linear dose-response);
- decreased MCH, increased MCV at ≥ 443 mg/kg bw/d;

Conclusion: Exposure of 222 mg/kg bw/d for 30 day is within the GVs (30 < C ≤ 300 mg/kg bw/d) for STOT RE 2, but due to lack of details it is not possible to compare the results with the classification criteria.

In a repeated-dose toxicity study (Krasavage, 1986, non-GLP, non-guideline), male rats were given 2-buthoxyethanol by gavage at doses of 222, 443, and 885 mg/kg bw/d for 5 d/week for 6 weeks.

Haematotoxicity:
- decrease in Hb concentration at all dose levels (-7% at 222 mg/kg bw/d; -22% at 443 mg/kg bw/d; -22% at 885 mg/kg bw/d);
- decrease in RBC counts at all dose levels (-12% at 222 mg/kg bw/d; -12% at 443 mg/kg bw/d; -34% at 885 mg/kg bw/d);
- decrease in MCHC at 443 mg/kg bw/d (-10%) and 885 mg/kg bw/d (-20%);
- increase in MCH at all doses (+6% at 222 mg/kg bw/d; +10% at 443 mg/kg bw/d; +18% at 885 mg/kg bw/d);
- increase in MCV at all dose levels (+8% at 222 mg/kg bw/d; +24% at 443 mg/kg bw/d; +48% at 885 mg/kg bw/d);
- blood in urine at ≤ 443 mg/kg bw/d.

Conclusion: The results of the study demonstrate that 2-buthoxyethanol, after repeated oral exposure for 30 days, at 222 mg/kg bw, thus within the GVs for oral exposure (30 < C ≤ 300 mg/kg bw/d), does not produce haematotoxic or other effects justifying classification as STOT RE 2.

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.

Inhalation exposure

In a non-guideline, non-GLP, repeat dose toxicity study (Gage, 1970), rats were exposed by inhalation (vapour, whole body) to 2-buthoxyethanol at concentrations of 0, 0.1; 0.24; 0.48; 1.2 mg/L (0, 20, 50, 100, 250 ppm), 6h/d, 5 d/week for 3 weeks.

Effects observed:
- adverse signs seen after 4 days at 1.2 mg/L, exposure to this concentration was not continued;
- no mortality;
- weight loss at 1.2 mg/L, animals lethargic;
- organs appeared normal.

Haematotoxicity:
- increased RBC osmotic fragility at ≥ 0.24 mg/L;
- initial haemoglobinuria at 1.2 mg/L;
- low Hb concentration and MCHC at 1.2 mg/L (no quantitative details reported)

Conclusion: The results of the study demonstrate that 2-buthoxyethanol after inhalation exposure for 15 days at 0.1, 0.24, 0.48, and 1.2 mg/L, thus within GVs for inhalation exposure (0.6 < C ≤ 3.0 mg/L/6h/d) does not produce haematotoxic or other effects justifying classification as STOT RE 2, although due to lack of reporting there is a considerable uncertainty in this conclusion.

In a non-guideline, non-GLP, repeat dose toxicity study (Mellon Institute of Industrial Research, 1956; cited in Carpenter *et al*., 1956) rats were exposed by inhalation (vapour, whole body) to 2-buthoxyethanol at concentrations of 0, 0.26, 0.52, 0.98, 1.5 (females only), and 2.1 mg/L (0, 54, 107, 203, 314 (females only), 432 ppm), 7 h/d, 5 d/ week for 6 weeks.

Effects observed:
- significantly increased mortality in females at ≥ 1.5 mg/L and males at 2.1 mg/L; no mortality at 0.98 mg/L;
- significantly higher liver and kidney weights at ≥ 0.52 mg/L;
- no further details reported.

Haematotoxicity:
- haemoglobinuria at ≥ 1.5 mg/L in females and in males at 2.1 mg/L (1 animal at 0.98 mg/L);
- significantly higher erythrocyte fragility at 0.26 mg/L at the end of the study (reversible within 24h);

Pathological effects:
- congestion and haemorrhage of lungs;
- congestion of most of abdominal viscera

Conclusion: The results of the study demonstrate that 2-buthoxyethanol, after inhalation exposure for 30 days at 0.26, 0.52, 0.98, 1.5 (females only) and 2.1 mg/L thus within GV for inhalation exposure (0.6 < C ≤ 3.0 mg/L/6h/d), produce some haematotoxic effects, but due to lack of details in the reporting (e.g. on Hb and RBC counts), it is not possible to compare

them with the classification criteria of STOT RE 2. However, it is noted that the increased mortality of females ≥ 1.5 mg/L and in males at 2.1 mg/L, could justify classification due to lethality.

In a non-guideline, non-GLP, repeat dose toxicity study (Mellon Institute of Industrial Research, 1956; cited in Carpenter *et al*., 1956), Guinea pigs were exposed by inhalation (vapour, whole body) to 2-buthoxyethanol at concentrations of 0, 1.8, 2.4 mg/L (0, 375 and 500 ppm), 7 h/d, 7 d/week for 30 days.

Effects observed:
- 2/10 male Guinea pigs died at 2.4 mg/L after 12 days, and 1/10 at 1.8 mg/L after 7 days;
- significantly lower body weight in females at 1.8 mg/L (not at higher concentration);
- lung haemorrhage and lung congestion at 1.8 mg/L;
- significantly higher kidney weights in females at ≥ 1.8 mg/L (< 10%)

Haematotoxicity (at ≥ 1.8 mg/L):
some effects seen but no no haematuria

Conclusion: The results of the study demonstrate that 2-buthoxyethanol, after inhalation exposure of Guinea pigs for 7 h/d, 7 d/week for 30 days at 1.8 and 2.4 mg/L, thus within GVs for inhalation exposure (0.6 < C ≤ 3.0 mg/L/6h/d), produced some haematotoxic effects, but due to due lack of details in the reporting (e.g. Hb and RBC counts) it is not possible to compare them with the classification criteria for STOT RE 2. However, it is noted that the increased mortality at 2.4 mg/L (20%) could justify classification due to lethality.

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

Oral route

In a study by Ghanayem *et al*. (2001; non-guideline, non-GLP, rats) at a dose of 250 mg/kg bw/d for 1, 2 or 3 days, no mortality of rats was observed, but there were considerable decreases of RBC counts (up to -80%), Hb (up to -21% in both sexes), HCT (up to 3-fold in both sexes), MCHC (up to -33%) and increases of MCV (up to -56-58%) and of MCH (up to -8%).

In Nyska *et al*. (1999, 2003), with doses of 250 mg/kg bw/d for up to 4 days, no mortality of rats was observed, but there were changes in the retina of both eyes such as multifocal haemorrhages within retinal pigment epithelium (RPE), in choriocapillaris, in inner and outer nuclear layers of the eye associated with degeneration and loss of cells, as well as retinal detachment. Positive expression of vascular cell adhesion molecule-1 (VCAM-1) occurred only in eyes of rats exposed to 3 and 4 doses and was localized in the iris (epithelium lining the posterior surface, anterior mesenchymal epithelium), ciliary processes (lining epithelium, stromal cells), and retina (hypertrophic retinal pigment epithelium). The appearance of VCAM-

1 immunostaining correlated with the development of thrombosis located in the same structures.

In a study by Grant *et al*. (1985; non-guideline, non-GLP, rat), at doses of 500 or 1000 mg/kg bw/d for 4 days, no mortality was observed, but there were considerable decreases of RBC count (day 1 after exposure: -23% at 500 mg/kg bw/d; -49% at 1000 mg/kg bw/d; day 8 after exposure: -10% at 500 mg/kg bw/d; -11% at 1000 mg/kg bw/d); with full recovery 22 days after exposure. There was also a reduced HCT (day 1 after exposure: -22% at 1000 mg/kg bw/d; full recovery 4 days after exposure), and Hb concentration (day 1 after exposure: -33% at 1000 mg/kg bw/d; full recovery 8 days after exposure).

In Lewis *et al*. (2006; non-guideline, non-GLP, rats) at a dose of 250 mg/kg bw/d for 4 days, no mortality was observed, but there were considerable decreases in RBC counts (-75%), significant increase in MCV (+54%), significant decrease in Hb concentration (no details reported), and a significant decrease in HCT (no details reported).

In a study by Ramot *et al*. (2007; non-guideline, non-GLP, rats), at a dose of ≥ 62,5 mg/kg bw/d for 4 days, no mortality was seen, but there was a significant decrease in RBC counts (> 10%), and significantly increased endothelial intercellular adhesion molecule-1 (ICAM-1; at ≥ 125 mg/kg bw/d in 6-w old rats and at ≥ 62.5 mg/kg bw/d in 12-w old rats). Histopathological findings were no intravascular thrombi in any of the 6-w old animals, but in 12-w old rats at 250 mg/kg bw/d, most prominent after 4 days of treatment. Most thrombi were in tail, nasal cavity, incisor teeth, and bone marrow (in 12-w old rats at ≥ 125 mg/kg bw/d). There were liver lesions in 12-w old rats at ≥ 125 mg/kg bw/d (multifocal hepatocellular necrosis), as well as thrombi also in the ciliary-body capillaries of the eye, auricle of the heart, and choroid plexus of the brain, and intracapillary microthrombi in renal glomeruli at 250 mg/kg bw/d.

In Ezov *et al*. (2002; non-guideline, non-GLP, rats), at a dose of of 250 mg/kg bw/d for 4 days, no mortality was seen, but there was a decrease in RBC counts (up to -30%), Hb concentration (up to -28%), HCT (up to -48%), MCHC (up to -62%), and a significant increase in MCH (up to +17%), MCV (up to +83%), as well as anisocytosis of erythrocytes (macrocytosis, schistocytosis and severe hypochromic ghost cells (linear dose-response)). There was also a significant increase in number of nucleated RBC prematurely released from bone marrow (linear dose/time-response); thrombosis (both sexes: coccygeal, vertebrae, heart; females only: femur, brain, liver, lungs, eyes) and infarction; renal tubular necrosis associated with Hb casts (haemoglobinuric necrosis); and splenic extramedullary haematopoiesis.

In a study by Redlich *et al*. (2004; non-guideline, non-GLP, rats), at a dose of of 250 mg/kg bw/d for 4 days, followed by 24 days observation period, no mortality of rats was observed, but there was congested and dilated blood vessels, presence of vascular occlusive thrombi in pulp of incisor and molar teeth (time-dependent increase in severity); progressive necrosis of odontoblasts with well-defined border between necrotic and vital cells in females; focal myocytic necrosis of tongue at day 2-4 with regenerative changes. At day 29, all changes disappeared completely, and the tissue appearance was normal.

In a study by Shabat *et al*. (2004; non-guideline, non-GLP, rats) at a dose of of 250 mg/kg bw/d for 4 days, no mortality was observed, but there was thrombosis and infarction of tail vertebrae in both sexes, females were more severely affected. The lesions were characterised

by extensive medullary fat necrosis, granulomatous inflammation, fibroplasia, growth plate degeneration, and new woven bone formation adjacent to necrotic bone trabeculae.

In Sivarao and Mehendale (1995; non-guideline, non-GLP, rats) at a dose of of 500 mg/kg bw7d for 7 days, no mortality of rats was observed. There was a decrease in HCT to 18.6 at 24h after treatment (baseline HCT: ~40.0); recovery by day 7.

In a study by Ghanayem *et al*. (1992, non-guideline, non-GLP, rats), at dose of of 125 mg/kg/d for 1, 2, 3, 6 or 12 consecutive days (with post exposure period: 24h after last dose), no mortality was observed, but there was a time-dependent increase in haemolysis of erythrocytes with decreased RBC count (-24% after 12 days), decrease in Hb concentration (> 10% after 6 days, -13% after 12 days), decreased HCT (-49% after 2 days, but recovered until end of study), and an increase in MCV (+20% after 12 days). There was an increased ATP concentrations and increased number of reticulocytes (linear dose-response up to 6 days), which slowly declined, but remained above control levels throughout the study (ATP after 12 days 29% higher; no. of reticulocytes after 12 days 57% higher).

In a study by Sleet *et al*. (1991; OECD TG 414; GLP), pregnant female rats were given 2-buthoxyethanol by gavage at doses 0, 30, 100, 200 mg/kg bw/d once a day for 3 days (GD 9–11; Test 1). In Test 2, doses 0, 30, 100, 300 mg/kg bw/d were given once a day for 3 days (GD 11–13); post exposure period 17 days. There was no mortality, but a reduction in body weight and increased spleen weights were seen at ≥ 100 mg/kg bw/d in both studies, and an increased kidney and liver weight at ≥ 200 mg/kg bw/d. Haematotoxic effects seen were severe haematotoxicity at ≥ 100 mg/kg bw/d, as well as dramatic reductions in circulating RBC, HTC, MCHC and Hb concentration after 24h (haemolytic anaemia). There were also increases in MCV, MCHC, reticulocytes and white blood cell count. By GD 20 the haematotoxic effects were nearly reversed. No quantitative details were reported and there was no information on erythrocyte morphology.

In Laifenfeld *et al*. (2010, non-guideline, non-GLP), mice were given 2-buthoxyethanol by gavage at a dose of 900 mg/kg bw/d for 7 days. Post exposure period: 14 days. No mortality was observed. Haematotoxic effects seen were a significant decrease in RBC counts (-23%), significant decrease in Hb concentration (-19%), and a significant increase in no. of reticulocytes (6.1-fold) Histopathological findings were a significantly increased splenic extramedullary haematopoiesis: increased numbers of haematopoietic precursors in the medullary red pulp with a predominance of erythroid lineage cells compared to myeloid precursors, and a significantly decreased bone marrow M/E ratio.

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.

Inhalation route

In Tyl *et al*. (1984; OECD TG 414, GLP), pregnant rats were exposed to 2-buthoxyethanol by inhalation at concentrations of 0, 0.12; 0.24; 0.48; 0.97 mg/L (vapour, 0, 25, 50, 100, or 200 ppm), 6 h/d for 10 days (GD 6–15); post exposure period: 6 days.

Effects seen:
- no mortality
- haematuria at ≥ 0.48 mg/L;
- pale, cold extremities with necrosis of the tail tip at 0.97 mg/L;
- weight loss at ≥ 0.48 mg/L

Haematotoxicity:
- significant reduction in RBC counts at ≥ 0.48 mg/L (-10% at 0.48 mg/L; -9% at 0.97 mg/L);
- significant increases in MCV and MCH at ≥ 0.48 mg/L (MCV: +11% at 0.48 mg/L; +30% at 0.97 mg/L; MCH: +11% at 0.48 mg/L; +25% at 0.97 mg/L);
- significant reduction in MCHC at ≥ 0.48 mg/L (-2% at 0.48 mg/L; -5% at 0.97 mg/L);
- significant increase in Hb concentration (+14%) at 0.97 mg/L;
- significant increase in HCT (+20%) at 0.97 mg/L

In Mellon Institute of Industrial Research (1952, non-guideline, non-GLP), rats were exposed by inhalation to 2-buthoxyethanol at concentrations of 0, and 0.97 mg/L (0, 200 ppm), 6-7 h/d for 10 days; post exposure period: 8 days.

No mortality observed.

Haematotoxicity after 4 days of exposure:
- significant decrease in RBC count (-50%);
- significant decrease in Hb concentration (-25%)

Haematotoxicity after 10 days of exposure:
- significant decrease in RBC count (> 50%);
- significant decrease in Hb concentration (> 25%)

After the post exposure period, the values recovered, but the RBC counts were still subnormal. The fagility values were normal.

In a study by Tyl *et al*. (1984; OECD TG 414, GLP) mice were exposed by inhalation (vapour) to 2-buthoxyethanol at concentrations of 0, 0.12; 0.24; 0.48; 0.97 mg/L (0, 25, 50, 100, 200 ppm), 6 h/d for 13 days (GD 6–18).

Effects observed:
- mortality at 0.97 mg/L (4/20) 3 days after exposure start;
- significantly lower body weight and body weight gain (linear dose-response) at 0.97 mg/L (8%)

Haematotoxicity (no quantitative details reported):

- no apparent haematological effects
- significant increases in Hb content and HCT at 0.48 mg/L but increase was not significant at 0.97 mg/L

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.

Dermal route

In Bushy Run Research Center (1989; non-guideline, non-GLP) male and female New Zealand White rabbits were exposed via occlusive dermal application to 2-buthoxyethanol at concentrations of 0, 5, 25, 50, 100% (corresponding to 45, 225, 450 and 900 mg/kg bw/d) 6 h/d for 9 days (dosed for 5 days, no dosing for 2 days and then dosed for further 4 days). Post exposure period: 14 days.

Effects observed:

- no mortality;
- decreased body weight gain in female rabbits treated with 100% 2-butoxyethanol throughout the study;
- no changes in any organ weight but dose related patchy colour change of the kidneys of 3 females at a concentration of 100% (undiluted).

Haematotoxicity:

- Hb in urine (2/4 males on day 2-5 at 100%; 4/5 females at 100% and 5/5 at 50% up to day 9);
- significantly decreased RBC counts, Hb concentration and MCHC and increased MCH on day 9 in females at a concentration of 100%;
- decreased HCT and increased MCV (recovered after post exposure period).

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**.

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