Special Report

No 7

Butoxyethanol Criteria Document

Including a Supplement for 2-Butoxyethyl Acetate

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ECETOC

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April 1994

European Centre for Ecotoxicology
and Toxicology of Chemicals

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ECETOC SPECIAL REPORTS

No.	Title
No. 1	Existing Chemicals, Guidance for Completing the ECC Data Set
No. 2	Existing Chemicals, Recommendations for Priority Setting
No. 3	Studies on Toxicokinetics and Macromolecular Binding of Styrene
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No. 4	1,3-Butadiene, Criteria Document.
No. 5	Environmental Health Criteria for Methylene Chloride.
No.6	Special Report : Interpretation - Evaluation of the Neurotoxic Potential of Chemicals in Animals.

PREFACE

This report has been prepared by ECETOC for use by the Commission of the EC DG V and its Scientific Expert Group. It contains an original review and assessment of toxicological data and quantitative risk assessments to provide a scientific basis for an occupational exposure limit for 2-butoxyethanol and 2-butoxyethyl acetate. Information on occurrence, production and use, exposure and uptake, and measurement techniques has been drawn largely from existing literature.

ECETOC Special Report No.7

ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals), 4 avenue Van Nieuwenhuyse, Bte. 6, 1160-Brussels, Belgium.

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Table of Abbreviations

ACGIH	American Conference of Governmental Industrial Hygienists
ADH	Alcohol dehydrogenase
ALDH	Aldehyde dehydrogenase
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AUC	Area under the blood concentration time curve
BAA	2-Butoxyacetic acid
bw	Body weight
CAS	Chemical Abstracts Service
CHO	Chinese hamster ovary
CNS	Central nervous system
d	day(s)
EAA	Ethoxyacetic acid
EC_{50}	Concentration that allows 50% cell formation
g.d.	Gestation day
Hb	Haemoglobin
Hct	Haematocrit
HGPRT	Hypoxanthine-guanine-phosphoribosyl transferase
i.v.	Intravenous
kg	Kilogram
KTV	Takgränsvärde (Sweden)
l	Litre
LC_{50}	Lethal concentration for 50% of the exposed animals
LD_{50}	Lethal dose for 50% of the exposed animals
l/min	Litre per minute
LOAEL	Lowest observable adverse effect level
MAK	Maximale Arbeitsplatzkonzentration (FRG)
MCHb	Mean cell haemoglobin
MCHC	Mean cell haemoglobin concentration
MCV	Mean cell volume
mg	Milligram
mg/m^3	Milligrams per cubic meter

ml	Millilitre
mM	Millimolar
mmol	Millimole
NGV	Nivågränsvärde (Sweden)
NIOSH	National Institute for Occupational Safety and Health
NOAEL	No observable adverse effect level
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
PBBK	Physiologically-based pharmacokinetic (Model)
ppm	Parts per million parts of air
RBC	Red blood cell (erythrocyte numbers)
RTECS	Registry of Toxic Effects of Chemical Substances
s.c.	Subcutaneous
REL	Recommended exposure level
STEL	Short term exposure limit
TLV	Threshold limit value
TWA	Time-weighted average
UDS	Unscheduled DNA synthesis
µmol	Micromole
WBC	White blood cell
wk	week

1. SUBSTANCE IDENTIFICATION

1.1. IDENTITY

2-Butoxyethanol has the chemical structure: $CH_3CH_2CH_2CH_2OCH_2CH_2OH$.
The IUPAC name is Ethylene glycol butyl ether and the Chemicals Abstracts Service 8th and 9th nomenclature name is Ethanol, 2-butoxy- . 2-Butoxyethanol is known by a large number of synonyms and trade names. These are detailed in Table I.

2-Butoxyethanol belongs to the family of ethylene glycol monoalkyl ethers represented by the general formula $R_1OCH_2CH_2OR_2$, where R_1 represents the alkyl (butyl) moiety and R_2 either H or acetate. For the purposes of this Criteria Document the name 2-butoxyethanol is used throughout.

Trade names and synonyms are also given in Table I.

Table I
2-Butoxyethanol - Synonyms and Trade Names

Name	Numbers	Synonyms	Trade Names
2-Butoxyethanol	EINECS[1] 203-905-0	2-n-Butoxyethanol 2-butoxyethanol Ethanol, 2-Butoxy	Buthyl Ethoxol® Dowanol EB® Butyl Cellosolve®
	EEC Classification number 603-014-00-0	Butyl Glycol	Ektasolve EB®
	CAS 111-76-2	Butyl Glycol Ether (BGE)	Jeffersol EB®
	RTECS KJ 8575000	Ethylene Glycol Butyl Ether (EGBE)	Butyl Oxitol®

1) European Inventory of Existing Chemical Substances

2-Butoxyethanol is classified and labelled in the EC as below.

EC Classification: Xn (Harmful) R20/21/22
Xi (Irritant) R37

EC Labelling: Symbol Xn

Risk Phrases: R20/21/22, R37
Harmful by inhalation, in contact with the skin and if swallowed.
Irritating to the respiratory system.

Safety Phrases: S24/25
Avoid contact with skin and eyes.

2. CHEMICAL AND PHYSICAL PROPERTIES

2-Butoxyethanol is a colourless liquid with a mild ether like odour. The odour threshold is 0.10 ppm (Amoore and Hautala 1983). It is soluble in water and most organic solvents. Other chemical and physical properties are listed in Table II.

2.1. CONVERSION FACTORS

1 ppm = 4.91 mg/m^3 (20°C; 1014 h Pa)
1 mg/m^3 = 0.204 ppm (20°C; 1014 h Pa)

Table II
Chemical and physical properties of 2-butoxyethanol [1)]

Property	Value
Molecular weight	118.2
Molecular formula	$C_6H_{14}O_2$
Specific gravity at 25°/4°C	0.898
Evaporation rate (butyl acetate = 1.00)	0.1
Boiling point °C	170.8
Freezing Point °C	-77
Vapour pressure (hPa at 25 °C)	1.17
Refractive index	1.417
Flash point (°C), closed cup	62
Autoignition temperature (°C)	238
Flammability limits (volume % in air)	1.10 - 12.7
Water solubility	Soluble
Vapour density (air = I)	4.1
ppm in saturated air (25°C)	1,200
Surface tension (mN/m at 25°C)	27.4

[1)] Adapted from Rowe and Wolf (1982).

3. OCCURRENCE

3.1. EMISSIONS

No data available

3.2. OCCURRENCE IN THE WORKPLACE

Exposure to 2-butoxyethanol occurs during production and during the end use of formulated products; see Section 5.1 for the available details.

3.3. BACKGROUND ENVIRONMENT

2-Butoxyethanol was listed as a contaminant found in drinking water samples analysed between September 1974 and January 1980 in a survey of US cities. No concentrations were given (Lucas, 1984).

2-Butoxyethanol was detected at a concentration of 23 ppb in one of 7 groundwater samples collected in February 1974 near the "Valley of the Drums", Kentucky, a contaminated site (Stonebreaker and Smith, 1980).

In 1980 2-butoxyethanol was detected in Hayashida River water (Matubara area in Tatsuno City, Hyogo prefecture) at concentrations of 1,310 and 5,680 ppb. The river water was contaminated with effluents from the leather industry. It was one of the most contaminated rivers in Japan. The two figures represent levels detected after steam distillation and vacuum distillation respectively (Yasuhara *et al*, 1981).

4. PRODUCTION AND USE DATA

4.1. PRODUCTION

2-Butoxyethanol is the most widely produced glycol ether with a production capacity in the European Community of approximately 70,000 tonnes per year . It is usually synthesised by a reaction of ethylene oxide with butyl alcohol. Ethylene glycol monoalkyl ethers are not formed as pure compounds but must be separated from the monoethers of diethylene glycol, triethylene glycol and the higher glycols. Temperature, pressure, reactant molar ratios, and catalysts are selected to give the product mix desired.

4.2. USE

In most applications 2-butoxyethanol is used because it is an excellent coupling agent. It is a small yet vital component of many water borne coating and cleaning formulations, allowing reformulation from volatile organic hydrocarbon solvents to water based systems. It is used in the formulation of many industrial and consumer products such as inks, coatings, cleansers, and fuel additives and as a solvent in surface coatings such as paints, spray lacquers, quick-dry lacquers, enamels and varnish removers. 2-Butoxyethanol is employed as an intermediate in 2-butoxyethanol acetate production, as a component in herbicides and some specialised automotive brake fluids.

5. QUANTITATIVE INFORMATION ON EXPOSURE AND UPTAKE

5.1. EXPOSURE LEVELS OF THE WORKPLACE

5.1.1. Number of workers potentially exposed

During production a few people are exposed to 2-butoxyethanol. Typically there are no personnel working constantly on the plant, only occasional visits from fitters, engineers and other technical staff take place. There is the potential for exposure to the chemical in control rooms but this is minimal (2 to 4 people per shift per production facility). There are several plants producing 2-butoxyethanol in the European Community.

In the USA, the National Occupational Exposure Survey (NOES) estimated that during the period 1981-1983, approximately 1.7 million workers were occupationally exposed to 2-butoxyethanol and 124,000 workers were occupationally exposed to 2-butoxyethanol acetate. Among industries labelled by the four digit Standard Industrial Code, 375 were identified as having workers potentially exposed to 2-butoxyethanol, and 104 were identified as having workers potentially exposed to 2-butoxyethanol acetate. NOES identified 222 occupations as having workers potentially exposed to 2-butoxyethanol, and 62 occupations as having workers potentially exposed to 2-butoxyethanol acetate (NIOSH, 1983).

5.1.2. Quantitative exposure data

The potential occupational exposure routes are inhalation and dermal (see also 7.3). Manufacturers of 2-butoxyethanol monitor production areas to ensure that occupational exposure standards are met. The results of such monitoring show very low workplace levels. For example, personal exposures at a number of production sites during 1988-1993 gave results in the a range 0.1-1.6 ppm and a mean of typically 0.13 ppm (data received from CEFIC, 1993).

The main reason for such low figures is that the plants on which 2-butoxyethanol is manufactured are designed to contain the primary feedstock, ethylene oxide. Data from production areas are therefore not a reliable indication of the level of the majority of occupational exposures to 2-butoxyethanol and to formulations containing 2-butoxyethanol during use.

There are a few reports of quantitative workplace (non production area) exposure studies. Recently, an analytical method for 2-butoxyethanol has been developed and used to determine systemic dose after exposure to formulated products by automobile and window cleaners (Vincent *et al*, 1993). The formulated cleaning products contained 2-butoxyethanol at concentrations ranging from 1 - 21%. The results indicate that inhalation exposure was a minor component of the systemic dose (highest mean air concentration about 5 ppm; mean air concentration 0.3 - 2.3 ppm), and that dermal absorption of the liquid formulation was the major contributor.

Veulemans *et al* (1987) examined 2,654 air samples from 336 different plants in Belgium for the presence of various glycol ethers. Though most exposure levels were below the current hygiene standards, in approximately 25% cases these levels were exceeded and sometimes extreme values were obtained (up to 370 ppm = 1,775 mg/m^3). Values at painting and printing facilities ranged from 3.4 - 93.6 mg/m^3 (0.7 - 19 ppm) and 1.5 -17.7 mg/m^3 (0.3 - 3.6 ppm) respectively. A median-value from car repair areas was 5.9 mg/m3 (1.2 ppm) (see also 7.3.3.).

5.2. PERSONAL MONITORING

Where data is available this is given in Section 5.1.2

5.3. ENVIRONMENTAL LEVELS

See Section 3.3

6. MEASUREMENT TECHNIQUES AND ANALYTICAL METHODS

6.1. AT THE WORKPLACE

The major methods for the analysis of 2-butoxyethanol, its acetate and other glycol ethers rely upon adsorption of the solvent onto a suitable adsorbent. The adsorbent can be charcoal (from a variety of sources) or a proprietary substance such as Tenax®. In all cases a measured volume of sample air is drawn through a glass or metal tube packed with adsorbent. The collected vapours are desorbed by a suitable solvent and the solution is analysed with a gas chromatograph. Full details of the available methods are given in the Handbook of Occupational Hygiene (instalment 15), UK-HSE (1984a) and NIOSH (1990) which details method 1403 for 2-butoxyethanol. The limit of detection of such a method varies upon the volume of air drawn over the adsorbent, and the nature of the adsorbent. Tenax® tubes give the lowest limit of detection, typically 0.1 ppm v/v, whereas charcoal systems give a limit of detection between 0.2 and 2 ppm. The methods are suitable for sampling over periods between 10 minutes and 8 hours.

6.2. ENVIRONMENTAL MONITORING

The principle methods given above for the occupational exposure monitoring can be adapted for the environmental monitoring for 2-butoxyethanol and its acetate (NIOSH, 1990).

6.3. BIOLOGICAL MATERIALS

No specific methods for the detection of 2-butoxyethanol in biological tissues are detailed in the literature. For the analysis of butoxyacetic acid in urine either fluoroanhydride derivatisation after extraction of the tetrabutylammonium ionpair (Smallwood *et al*, 1984), extractive alkylation with pentafluorobenzyl bromide (Johanson and Johnsson, 1991) or diazomethane derivatization after urine lyophilisation (Groeseneken *et al*, 1989) were used or, more recently, a combination of these methods.

The use of other methods is described in Section 7.3.4.

7. TOXICOLOGY

7.1. TOXICOKINETICS

7.1.1. General Aspects

2-Butoxyethanol is well absorbed after ingestion, skin contact and inhalation exposure. Studies in F344 rats indicate that approximately 70-80% of an oral dose is absorbed from the gut and recovered in urine within 48-72 h. Quantitative data on absorption from the human alimentary tract are lacking, although 2-butoxyethanol or its metabolites have been detected in urine following accidental or deliberate ingestion of certain household cleaning products.

7.1.2. Uptake

In vitro investigations with undiluted 2-butoxyethanol under occlusive conditions showed an absorption rate of 1.67 μmol/cm^2/h (198 μg/cm^2/h) across human abdominal skin (Dugard *et al*, 1984.). Bartnik *et al* (1987) extended these observations, and compared dermal absorption of neat and aqueous (3.5%, 10%) 2-butoxyethanol across skin samples from a number of species *in vitro*. Skin from hairless rats, pig skin (dorsum) and human skin (forearm) were used in these studies, and measurements conducted under open and semi-occluded conditions. The initial absorption (measured over six hours) of pure 2-butoxyethanol was slower than for aqueous solutions. Nevertheless total uptake after 16 h was greater for the neat material. Absorption by rat skin was some 2-fold greater than for pig - and human skin. The authors did not report absolute absorption rates, but a value of 17.3 μg/cm^2/h may be derived for human tissue under semi-occluded conditions. This is approximately 11-fold less than that reported by Dugard *et al*, 1984.

Approximately 20-23% of a non-occluded dose of radiolabelled 2-butoxyethanol (200 mg) was recovered in urine over 48 h following application to intact rat skin (12 cm^2, ventral surface) (Bartnik *et al*, 1987). The maximum level of radioactivity in blood was achieved within 2 h. The majority (95%) of the label was excreted within the first 24 h. There was no marked sex difference. By comparison with the urinary excretion seen after subcutaneous injection (79.6% over 48 h) the authors calculated a corrected percutaneous absorption of 24-29% for the exposure conditions employed. It is likely that under non occluded conditions the majority of the applied dose would have evaporated before absorption could occur.

Dermal absorption of 2-butoxyethanol by female guinea pigs was investigated by Johanson and Fernström (1986) after application of 1 ml of neat material to approximately 3 cm^2 of skin under occluded conditions. Absorption was estimated as 0.25 μmol/min/cm^2, equivalent to 15 μmol/cm^2/h. Absorption of aqueous solutions of 2-butoxyethanol was reported in a subsequent investigation (Johanson and Fernström, 1988) in which guinea pigs were exposed to 5, 10, 20, 40 and 80% aqueous solutions for 2 h under occlusive conditions. Uptake of neat 2-butoxyethanol was then determined after a 2 h recovery period. Absorption rates were similar for all aqueous solutions (range 0.52 - 0.73 mg/cm^2/h) which were 2 fold higher than for undiluted liquid (0.27 mg/cm^2/h). Marked variations in blood levels were

recorded during the second phase of this study which may reflect differences in dermal hydration as a consequence of the earlier treatment with aqueous 2-butoxyethanol.

Johanson *et al* (1988) studied direct dermal absorption in 5 volunteers who immersed 2 or 4 fingers (equivalent to 79-189 cm^2) in undiluted 2-butoxyethanol for 2 h. Blood samples were collected for up to 4 h after the end of the exposure period and analysed (for 2-butoxyethanol) by gas chromatography. The rate of absorption varied between 7-96 nmol/min/cm^2, with a geometric mean of 20 nmol/min/cm^2. This figure is comparable to the 24-28 nmol/min/cm^2 uptake reported by Bartnik *et al* (1987) and Dugard *et al* (1984), respectively for human skin *in vitro*. The average 24 h urinary excretion rate of 2-butoxyacetic acid was 17% (2.5-39%) of the total uptake. The authors estimated the continuous dermal exposure of 4 fingers during 2 h to be equivalent to an inhalation chamber exposure of 20 ppm for 2 h, based on a previous inhalation experiment where the respiratory uptake averaged 10.1 μmol/min during exposure to 20 ppm under light physical exercise (Johanson *et al*,1986a).

A study to investigate the dermal absorption of 2-butoxyethanol vapour was conducted by Johanson and Boman (1991) who exposed four volunteers to an atmosphere of 50 ppm for 2 h. The subjects were dressed in shorts to maximise dermal exposure, and were fitted with air breathing apparatus to minimise inhalation uptake. 2-Butoxyethanol levels in blood reached approximately 6.3 μmol/l during the second hour of exposure, and an uptake rate of 32 μmol/min was estimated. Dermal absorption was reported to increase (concentration in blood 12 μmol/l, uptake rate 44 μmol/min) when the investigation was repeated under humid conditions (33°C, 71% humidity). Although there were no statistically significant differences between the two results, increased dermal hydration may be the explanation for any differences in permeability. The authors concluded that the dermal absorption was significant following vapour exposure and may account for up to 75% of the internal dose of 2-butoxyethanol. This finding appears to be contrary to the commonly held view that the internal dose contribution of dermal absorption of organic solvents is relatively minor compared to that due to inhalation of vapour.

The inhalation absorption of 2-butoxyethanol was measured by Johanson *et al* (1986a) in seven volunteers exposed to 20 ppm vapour and undertaking light physical exercise for 2 h (pulmonary ventilation rate 22.6 l/min). The concentration of 2-butoxyethanol in blood reached a plateau of 7.4 μmol/l within 1-2 h of the start of exposure, and the respiratory uptake was estimated as 10.1 μmol/min or 57% of the dose. In a subsequent study, Johanson and Boman (1991) measured respiratory uptake in four volunteers who were exposed, by a face mask, to 50 ppm 2-butoxyethanol for 2 h (pulmonary ventilation rate 8.3 l/min). Blood levels attained a steady state of 2.4 μmol/l during the second hour of exposure, and respiratory uptake rate was estimated as 9.9 μmol/min. Comparison of the two studies suggested a greater uptake of 2-butoxyethanol in the former despite the lower exposure concentration, which could only be partially explained by the difference in pulmonary ventilation rate. Johanson and Boman (1991) concluded that the earlier protocol had probably allowed simultaneous respiratory and dermal uptake to occur.

In a similar, unpublished study, Van Vlem (1987) investigated the uptake of 2-butoxyethanol vapour following inhalation exposure to 12.6 or 25.2 ppm, and reported absorption of approximately 68% of the dose. The degree of absorption was unaffected by moderate exercise.

7.1.3. Distribution

2-Butoxyethanol is distributed via the blood stream to all tissues. At high dose levels small amounts of unchanged 2-butoxyethanol may be found in urine, but more commonly it is excreted as its metabolites (primarily 2-butoxyacetic acid) or exhaled as carbon dioxide.

7.1.4. Biotransformation and excretion

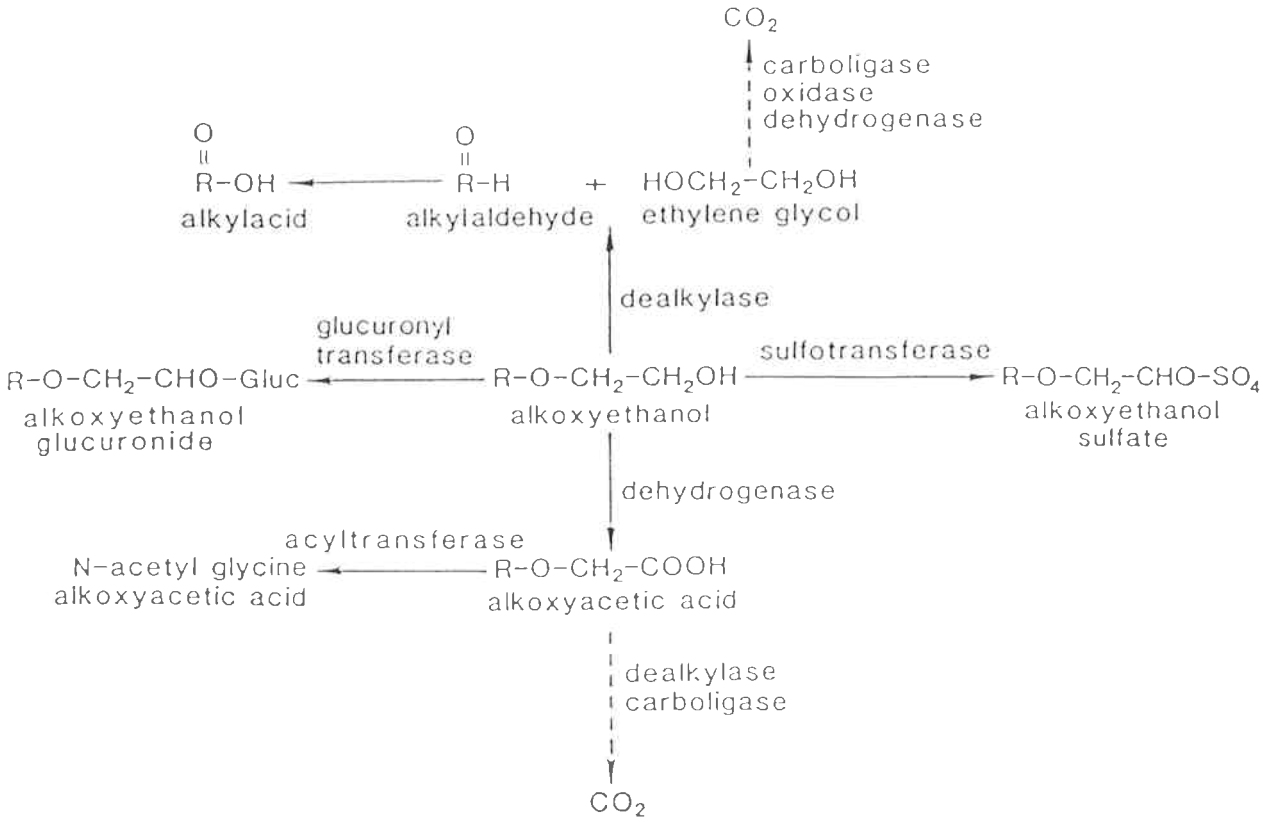
The major metabolite of 2-butoxyethanol is 2-butoxyacetic acid. A schematic diagram of the metabolism of glycol ethers in the rat is given in Figure I.

Ghanayem *et al* (1987a) administered a single oral dose (125 or 500 mg/kg bw) of (^{14}C)-labelled 2-butoxyethanol to male F344 rats (9-13 wk old), and followed metabolism over a 48 h period. At the lower dose, 70% of the label was excreted in urine within 48 h, while at the higher dose urinary excretion accounted for 40% over this same period. 2-Butoxyacetic acid was the predominant product formed, irrespective of dose level, and accounted for greater than 75% of the urinary metabolites detected 8, 24 and 48 h post treatment. Trace amounts of 2-butoxyethanol sulphate (approx. 3%) and unchanged 2-butoxyethanol (approx. 2%) were also detected, but only at the 8 h time point in urine from the low dose animals. Excretion of carbon dioxide was inversely related to treatment level (accounting for 18% of the dose at 125 mg/kg bw and 10% at 500 mg/kg bw), possibly reflecting saturation of metabolism at high dose levels. Biliary excretion in the first 8 h accounted for 8% of the dose at 500 mg/kg, with 2-butoxyethanol glucuronide the major metabolite, and lesser amounts of 2-butoxyacetic acid. Faecal excretion over 48 h was 2-3% of the administered material, irrespective of the dose. Radioactivity was found in all tissues 48 h after treatment. The relatively slow elimination was regarded by the authors as a consequence of high-dose saturation of the enzymes responsible for metabolism of 2-butoxyethanol. On the basis of these observations, the authors identified the following metabolic pathways for 2-butoxyethanol in the rat: oxidation to 2-butoxyacetic acid, conjugation of 2-butoxyethanol with glucuronide or sulphate.

In a subsequent study, Ghanayem *et al* (1987b) demonstrated that a large dose of (^{14}C)-labelled 2-butoxyethanol (500 mg/kg bw body weight, by gavage) was more completely metabolised by young (4-5 wk old) F344 rats in comparison with older (9-13 wk) animals. The young animals converted a higher proportion of an intubated dose of 500 mg/kg bw to carbon dioxide (22% compared with 10% in the older animals) possibly reflecting enhanced degradation of 2-butoxyacetic acid. The percentage of the administered dose excreted in the 24-h urine was higher in the young rats and there was a higher 2-butoxyethanol glucuronide: 2-butoxyacetic acid ratio.

Figure I Pathways of metabolism of 2-Butoxyethanol*

R = CH_3-$(CH_2)_3$-



* from Medinsky *et al* (1990)

In the younger rats a minor uncharacterised metabolite was found in the urine at levels equivalent to 6-8% of the dose, while in adult rats, this was present at levels below 1%. Lower levels of 2-butoxyethanol derived radioactivity were found in all tissues of young rats as compared with tissues of older animals. The authors proposed that these findings indicated an increase in the "activation/detoxification" index in older animals due to reduced catabolism and renal clearance of 2-butoxyacetic acid. The findings offered a metabolic basis for the increased susceptibility of older rats to the haematologic and secondary toxic effects associated with the administration for large doses of 2-butoxyethanol (see section 7.2.9).

Medinsky *et al* (1990) exposed male Fisher 344/N rats (11-12 wk old) to 2-butoxyethanol in the drinking water at concentrations 290, 860 and 2,590 ppm (equivalent to ~28, 47 and 140 mg/kg). No significant differences in excretion pattern was observed for the three doses, with 75-79% of the radioactivity excreted over 72 h. 2-Butoxyacetic acid accounted for 50-60% of the urinary metabolites (greater conversion at the lower dose), approximately 10% was present as ethylene glycol, together with lower amounts of 2-butoxyethanol glucuronide and traces of unchanged 2-butoxyethanol. Between 8-10% of the administered material was exhaled as carbon dioxide, which appeared to correlate with production of ethylene glycol. Ethylene glycol was a previously unreported metabolite, and was considered to arise as a consequence of the cytochrome P-450 mediated dealkylation of 2-butoxyethanol.

Although the formation of a 2-alkoxyacetic acid glycine conjugate has been reported in urine of rats exposed to methoxy- or ethoxyethanol, no evidence of 2-butoxyacetic glycinate was found in this study (Sabourin *et al*, 1993). On the basis of data obtained for other glycol ethers, the authors speculated that this metabolite could be produced at the expense of ethylene glycol in situations involving high dose/high dose-rate exposures.

The disposition of 2-butoxyethanol in experimental animals following dermal absorption was studied by Sabourin *et al* (1992a) in F344 rats (11-12 wk old). The test material was dissolved in acetone and applied under a semi-occlusive dressing at dose levels of 520, 1,530 and 2,530 μmol/kg. Ingestion was prevented by covering the treatment site with a perforated capsule. The animals were housed in metabolism cages for 72 h after dosing. Approximately 20-25% of the applied material was absorbed and metabolised, but no dose-related trend was apparent in the type or quantity of metabolites produced. Most (approximately 82%) of the absorbed radioactivity was excreted in urine, 3-4% as carbon dioxide with small amounts (2-4%) detected in the faeces. The urinary metabolites were identified as 2-butoxyacetic acid (66-70%), 2-butoxyethanol glucuronide (13-15%), ethylene glycol (4-6%) and around 5-13% in the form of uncharacterised compounds. 2-Butoxyacetic acid was the principle metabolite found in plasma. As with the oral studies reported by this group no 2-butoxyacetic acid glycinate was found in this investigation (Medinsky *et al*, 1990; Sabourin *et al*, 1993).

Sabourin *et al* (1992b) studied the metabolism of isotopically labelled 2-butoxyethanol by F344 rats (11-12wk) after inhalation exposure (4.3, 49 and 438 ppm over 6 h). Uptake, metabolism and excretion of 2-butoxyethanol were reported to be proportional to exposure

concentration in this investigation despite evidence of clear toxicity in animals at the highest exposure level (two out of four rats died, the survivors showed signs of haematuria). Between 64-76% of the absorbed dose was detected in urine during the 4 h following treatment. 2-Butoxyacetic acid (accounting for 36-43% of urinary metabolites) and ethylene glycol (8-16%) were the principle transformation products reported, but their appearance in urine decreased as the exposure concentration increased. 2-Butoxyethanol glucuronide was also present in an approximately dose-dependent manner (accounting for 3% of the metabolites at the low dose and 10% at the higher dose). Two unidentified metabolites were also present in trace quantities, and appeared to increase in relation to dose. Approximately 5-8% of the retained material was exhaled as labelled carbon dioxide. The authors suggested that metabolism of 2-butoxyethanol by pathways leading to the formation of ethylene glycol and 2-butoxyacetic acid appear to be readily saturated. The decreased production of these metabolites at high dose levels appeared to be compensated by an increased formation of the glucuronide. They suggest that glucuronidation is favoured under conditions which result in high internal concentrations of 2-butoxyethanol (i.e. high Km for the glucuronyl transferase enzyme).

Carpenter *et al* (1956) exposed rats, rabbits and guinea pigs to 2-butoxyethanol by inhalation at concentrations of 100-400 ppm in air and found 2-butoxyacetic acid in the 24 h urine at levels related to the exposure concentration. Butoxyacetic acid was also found in the urine of dogs exposed to 200 and 400 ppm but no dose relationship was identified in this instance. Although 2-butoxyacetic acid was not identified in the blood of dogs, rats or guinea pigs, it was found in the blood of rabbits following intravenous administration.

The disposition of 2-butoxyethanol in man is qualitatively similar to that reported in experimental animals, with 2-butoxyacetic acid the principle metabolite produced. Johanson *et al* (1988) found 2-butoxyacetic acid in urine following dermal exposure to 2-butoxyethanol. The excretion of this metabolite peaked approximately 5 h post-exposure, with a half life of 3.1 h. Of the 127-1,891 μmol of 2-butoxyethanol absorbed during the 2 h exposure period, between 8.7-313 μmol 2-butoxyacetic acid was excreted in urine over the subsequent 24 h. (2-Butoxyacetic acid excretion showed marked inter-and intra-individual variation, and was not dosely correlated with 2-butoxyethanol exposure).

When seven male volunteers were exposed to 20 ppm 2-butoxyethanol in air for 2 h under conditions of light exercise, 17-55% (mean 41%) of the absorbed dose was recovered in the urine as 2-butoxyacetic acid within 24 h. Less than 0.03% of the absorbed material was excreted as unchanged 2-butoxyethanol. 2-Butoxyacetic acid was detected in urine shortly after exposure ceased, reaching a maximum within 2-10 h. There was wide inter-and intra-variation in the excretion rate and concentration of 2-butoxyacetic acid in urine at any given time (Johanson *et al*, 1986a).

Venous blood samples from 5 human volunteers exposed to 20 ppm of 2-butoxyethanol for 2 h showed the presence of 2-butoxyacetic acid in all samples (22-60 μM). The half life of 2-butoxyacetic acid in blood was 4 h. The renal clearance rate was low (22-39 ml/min) indicating a high plasma protein binding for 2-butoxyacetic acid and a low tubular secretion

(Johanson and Johnsson, 1991). A previously unidentified glutamine conjugate of 2-butoxyacetic acid (a detoxification pathway), recently reported as being present in the urine of exposed individuals (Rettenmeier *et al*, 1993), but not detected in rodent studies may explain the poor correlation between exposure and excretion in human studies, e.g. as seen in the studies of Johanson *et al* (1986a).

Römer *et al* (1985) reported that orally-administered ethanol competitively inhibited ADH and ALDH and slowed the biotransformation and the elimination of 2-butoxyethanol by rats. A similar inhibitory effect of ethanol on the 2-butoxyethanol elimination was also found in perfused rat liver (Johanson *et al*, 1986b).

Further investigations into the enzymology of 2-butoxyethanol metabolism were conducted by Ghanayem *et al* (1987c), who studied the effects of inhibition ADH or ALDH on the metabolism of (^{14}C)-2-butoxyethanol by adult (9-12 wk) male F344 rats. The animals received an i.p. injection of pyrazole or cyanamide before the oral administration of a single dose of 2-butoxyethanol (500 mg/kg bw by gavage). Pyrazole, an inhibitor of ADH, decreased excretion of labelled carbon dioxide (from 11% to 6%) and markedly increased the urinary excretion of 2-butoxyethanol-glucuronide (accounting for up to 85% of the radioactivity present in urine). Between 8-19% of the administered material was present in urine as 2-butoxyethanol-sulfate, which was not detected in the absence of pyrazole treatment. Urinary excretion of 2-butoxyacetic acid was decreased from 75-90% of the dose in control rats to 10% or less after pyrazole pretreatment. Cyanamide (an inhibitor of ALDH) also greatly decreased 2-butoxyacetic acid excretion (13% of dose at 8 h), with the majority (77%) of urinary label being in the form of 2-butoxyethanol-glucuronide and 10% as the sulphate.

7.1.5. Physiologically-based pharmacokinetic [PBPK] model.

PBPK models simulate physiological and biochemical factors which influence metabolism and fate of chemicals in the body. They permit extrapolation from one species to another (i.e. animal to man), and may remove the need for default uncertainty assumptions in human hazard evaluations based upon animal NOEL information. A number of such models have been described for 2-butoxyethanol.

A human PBPK model for inhalation of 2-butoxyethanol was first reported by Johanson (1986) and subsequently modified and extended by Corley *et al* (1993), Corley and Bormett (1993) and Shyr *et al* (1993) to include additional routes of exposure and metabolism. The model of Corley *et al* (1994) and Corley *et al* (1993) (Fig. II) is used extensively in this hazard evaluation.

The model successfully describes the disposition of 2-butoxyethanol and 2-butoxyacetic acid following a number of exposure scenarios. For example, rats exposed at the no effect concentration of 25 ppm 2-butoxyethanol were predicted to show an Area Under the blood concentration time Curve (AUC) for 2-butoxyacetic acid of 261 μmol h/l; an equivalent blood level of 2-butoxyacetic acid in man was predicted after 8 h exposure at the slightly lower level of 22 ppm 2-butoxyethanol.

The model also indicated that rats exposed at the NOEL of 25 ppm would exhibit a peak concentration of 33 μM 2-butoxyacetic acid following 8 h exposure to 2-butoxyethanol, whereas an equivalent level of the haemolytic metabolite in human blood would require a relatively higher inhalation exposure to 29 ppm 2-butoxyethanol. This concentration (33 μM) of 2-butoxyacetic acid is 240 fold less than the 8,000 μM concentration reported to cause minimal haemolysis in human RBC *in vitro.* (Ghanayem, 1989).

Taken together, the above arguments suggest that human beings are unlikely to show evidence of haemolytic changes following exposure to 22 ppm 2-butoxyethanol over an 8 h work period.

The PBPK model is also useful in assessing the relative contribution of dermal and inhalation absorption of 2-butoxyethanol vapour. For example, Johanson and Boman (1991) reported that for man exposed to 50 ppm 2-butoxyethanol vapours, either dermally or by inhalation, dermal exposure alone resulted in approximately 2-fold higher blood 2-butoxyethanol levels than inhalation exposure alone (mouth only exposure). This result suggested to these authors that dermal uptake of 2-butoxyethanol accounts for about 75% of total uptake during whole body exposure to 2-butoxyethanol vapour. These findings appear unusual, based on the anatomical structure and physiological properties of the lung and skin with respect to vapour exchange (cardiac output: lung 100%, skin 3%; surface area: lung 30-100 m^2, skin 1.9 m^2). The authors assume that blood samples collected from the finger-tip prick represented "general" or "systematic arterial" blood. If, in contrast, this blood is assumed to represent venous blood drained from the skin prior to dilution with pooled venous blood, and assuming a more realistic permeability coefficient, the Corley *et al* model (1994) predicts that the dermal route contributes no more than 20% of to the systemic dose.

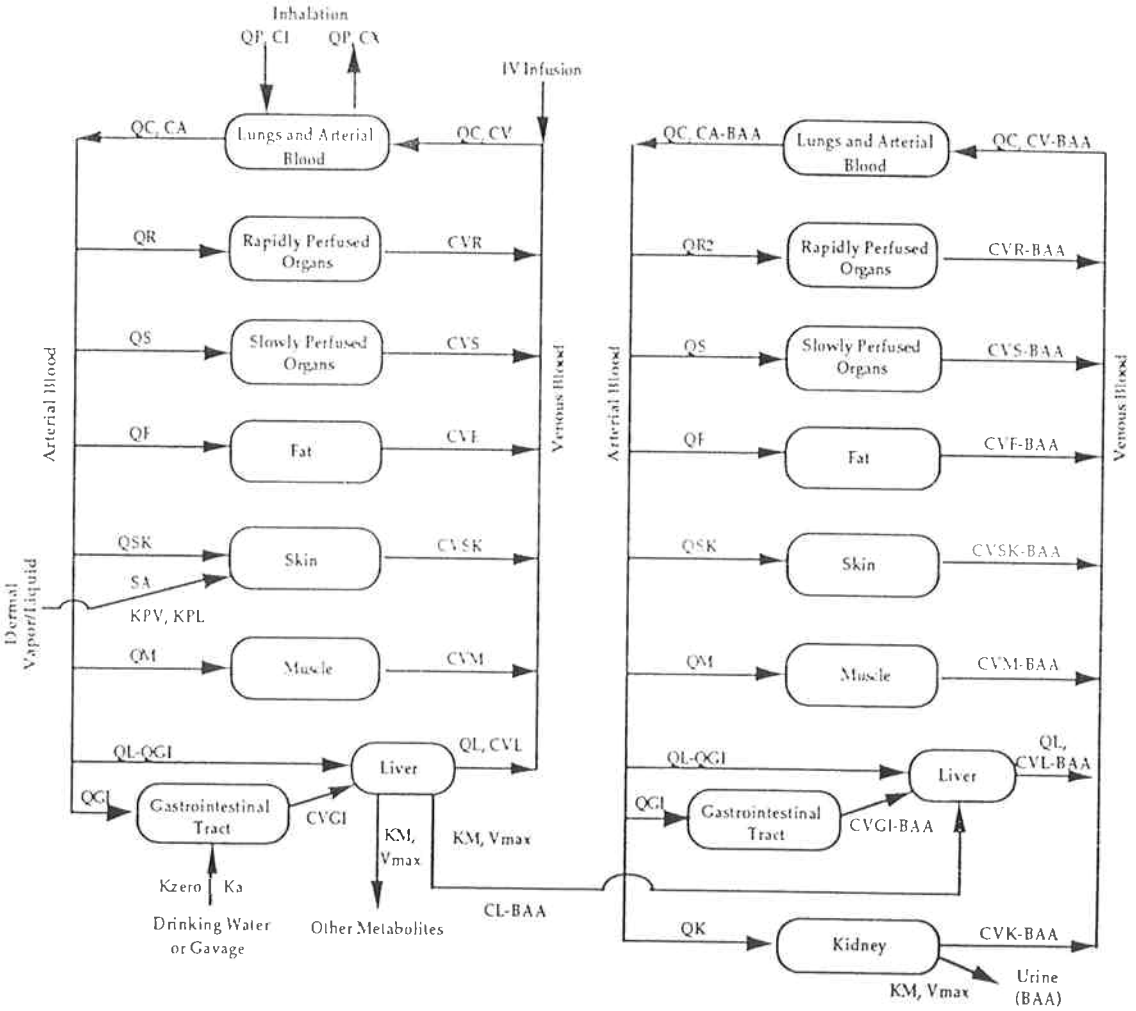
Nevertheless, even if the actual dermal contribution to systemic uptake of 2-butoxyethanol during whole body exposure of man accounts for up to 75% of the total uptake, the actual measured peak blood concentrations of 2-butoxyethanol seen after whole body exposure to 20 ppm for 2 h were only 7.4 μM (Johanson *et al*, 1986a). Exposure to 50 ppm (2 h) resulted in peak concentrations of 2-butoxyethanol of only 2.4 μM (mouth only) and 6.3 μM (skin only), respectively (Johanson and Boman, 1991) (see 7.1.2). Furthermore, human venous blood samples showed 2-butoxyacetic acid concentrations in the range of 22-60 μM following whole body exposure to 20 ppm 2-butoxyethanol for 2 h (Johanson and Johnsson, 1991; see chapter 7.1.4). This is in agreement with the PBPK model predicting human 2-butoxyacetic acid blood concentrations of approximately 30 μM at similar exposure levels (Corley *et al*, 1994).

In summary, the available experimental data in man and the predictions of the PBPK model indicate irrespective whether dermal uptake is the major contributor to the overall systemic uptake in man during whole body exposure to 2-butoxyethanol vapour that the effective blood concentration of the haemolytic metabolite 2-butoxyacetic acid following human exposure at 20 ppm is several hundred fold below those needed to cause minimal haemolysis of human red blood cells *in vitro.*

Figure II

PBPK-Model of 2-Butoxyethanol and 2-Butoxyacetic Acid (Corley *et al*, 1993)

Model for 2-Butoxyethanol (BE) Model for 2-Butoxyacetic Acid (BAA)



KEY:

CI.....Concentration BE in Inhaled Air (mg/l)
CX.....Concentration BE in Exhaled Air (mg/l)
QP.....Alveolar Ventilation (l/hr)
QC.....Cardiac Output (l/hr)
Qi.....Blood Flow to "i" Tissue (l/hr)
CA.....Arterial Blood Concentration BE (mg/l)
CA-BAA.....Arterial Blood Concentration BAA (mg/l)
CVi.....Venous Blood Concentration BE draining "i" Tissue (mg/l)
CVi-BAA.....Venous Blood Concentration BAA draining "i" Tissue (mg/l)
Kzero.....Zero-Order Rate Constant for Absorption of BE From Water
Ka.....First-Order Rate Constant for Absorption of BE From Gavage Dose (hr^{-1})
KM.....Michaelis Constant for Saturable Process (mg/l)
Vmax.....Maximum Velocity for Saturable Process (mg/hr)

7.1.6. Summary and conclusion

Dermal and inhalation exposure are likely to account for the principle human contact with 2-butoxyethanol.

The pharmacokinetics of 2-butoxyethanol have only been studied in detail in male F344 rats. In this strain the favoured metabolic pathway for lower systemic doses following inhalation, dermal or oral exposure involves conversion by ADH and ALDH to 2-butoxyacetic acid, which is rapidly excreted in the urine. There is an approximate first order relationship between 2-butoxyethanol exposure and 2-butoxyacetic acid production, up to a threshold above which metabolism to 2-butoxyacetic acid is saturated. Other minor urinary metabolites include ethylene glycol, 2-butoxyethanol glucuronide, 2-butoxyethanol sulphate and small amounts of unchanged 2-butoxyethanol.

Some of the absorbed 2-butoxyethanol is completely metabolised to carbon dioxide, which is excreted in the expired air. At higher systemic doses the ALDH and ADH pathways appear to become saturated and glucuronide conjugation features more prominently, but even so butoxyacetic acid remains the major metabolite.

F344 rats show some age differences in the metabolism of 2-butoxyethanol, the younger animals being more adept at excreting and/or further metabolising 2-butoxyacetic acid.

The metabolism of 2-butoxyethanol in other species, including human beings, is less well characterised, although volunteer studies show that 2-butoxyacetic acid is the major urinary metabolite for systematically absorbed 2-butoxyethanol in man. It has been detected following inhalation, dermal or oral exposure, but it should be noted that pronounced inter-individual variation is present in the extent and rate of this conversion.

A PBPK-model using blood concentrations of 2-butoxyacetic acid as internal dose surrogate for interspecies comparison between rat and man indicates that at an inhalation exposure standard of 20 ppm haemolysis is not likely to occur in man.

7.2. TOXICODYNAMICS

7.2.1. Acute Toxicity

7.2.1.1. Oral administration

Carpenter *et al* (1956) reported the oral LD_{50} on three different strains of rats determined over a period of seventeen years. They found that young rats were more resistant to single acute oral doses than one year old animals. There was little difference in the LD_{50} value when the solvent was dosed as a solution or undiluted. Details of the doses are given in the acute toxicity summary table (Table III).

Sluggishness, ruffling of coats and narcosis and haemoglobinuria followed the administration of doses close to the LD_{50}. At necropsy animals show haemorrhagic lungs, oedema, mottled livers and congested kidneys. Early deaths were attributed to a narcotic effect while delayed deaths were caused by lung and kidney damage which was probably secondary to haemolysis.

Fischer rats (> 5 males per group; of ages 4 - 5; 9 - 13; 22 - 26 or 69 weeks) received single oral doses of 32, 63, 125, 250 or 500 mg/kg bw. Dose and age related haemolytic effects were found. The oldest rats showed these effects at all levels down to 32 mg/kg bw, whereas all the younger rats responded only at 250 and 500 mg/kg bw (Ghanayem *et al*, 1987b).

Younger animals also show a lower internal dose of the predominantly active metabolite butoxyacetic acid (see 7.1.4.).

Table III surveys acute oral toxicity data.

Table III
Acute oral toxicity of 2-butoxyethanol

Species / Strain / Sex / Age/weight	Dosage mg/kg bw	Effect / Observations	Reference
Rat/Carworth - Wistar/M/30-60 g	3,000	LD_{50}	Carpenter *et al* (1956)
Rat/Carworth - Wistar/F/30-60 g	2,300	LD_{50}	Carpenter *et al* (1956)
Rat/Wistar/M/90-120 g	1480	LD_{50}	Carpenter *et al* (1956)
Rat/Sherman/M/90-120 g	2,800	LD_{50}	Carpenter *et al* (1956)
Rat/Carworth - Wistar/M/90 - 120 g	1,900	LD_{50}	Carpenter *et al* (1956)
Rat/Sherman/M/90-120 g	2,600	LD_{50}	Carpenter *et al* (1956)
Rat/Carworth - Wistar/M/90- 120 g	2,400	LD_{50}	Carpenter *et al* (1956)
Rat/Carworth - Wistar/M/90-120 g	2,100	LD_{50}	Carpenter *et al* (1956)
Rat/Carworth - Wistar/F/90- 120 g	2,800	LD_{50}	Carpenter *et al* (1956)
Rat/Sherman/F/90-120 g	1,600	LD_{50}	Carpenter *et al* (1956)
Rat/Sherman/F/90-120 g	2,300	LD_{50}	Carpenter *et al* (1956)
Rat/Carworth - Wistar/F/90-120 g	1,600	LD_{50}	Carpenter *et al* (1956)
Rat/Carworth - Wistar/M/335- 460 g	560	LD_{50}	Carpenter *et al* (1956)
Rat/Carworth - Wistar/F/260- 320 g	530	LD_{50}	Carpenter *et al* (1956)
Rat/Fischer/M/Aged 4-5, 9-13, 22-26 or 69 weeks	32, 63, 125, 250 or 500	Dose and age related haemolytic effects observed. Older rats demonstrated effects down to 32 mg/kg bw. Younger rats showed effects at 250 and 500 mg/kg bw.	Ghanayem *et al* (1987b)
Rat/Wistar Derived/M/	1,790	LD_{50} (Average from 5 laboratories)	Weil and Wright (1967)
Mouse/M/20-30 g	1,230	LD_{50}	Carpenter *et al* (1956)
Guinea Pig/M + F/200-300 g	1,200	LD_{50}	Carpenter *et al* (1956)
Rabbit/M/1,500-3,000 g	370	LD_{50}	Carpenter *et al* (1956)
Rabbit/M/2,700-3,200 g	320	LD_{50}	Carpenter *et al* (1956)
Rabbit/M/1,500-3,000 g	0.1 - 2.0*	Death occurred within 30 h. No effects at 0.1 or 0.5 ml/kg bw	Gross cited in UK-HSE (1984b)

* ml/kg bw

7.2.1.2. Dermal administration

In male and female Wistar rats no mortality was reported after non-occlusive dermal administration of 200 - 500 mg/kg bw. The lowest observable effect level to cause haemolytic effects was 260 mg/kg bw via this administration route under these conditions. No effects were found at 200 mg/kg bw (Bartnik *et al*, 1987). In guinea pigs a dermal LD_{50} of 200 - 300 mg/kg bw was reported with values of 210 mg/kg bw for intact skin and 270 mg/kg bw for abraded skin (Roudabush *et al*, 1965).

In 3-5 months old rabbits 24 h of exposure to the concentrated material under occlusive conditions resulted in an LD_{50} of 400-500 mg/kg bw (Carpenter *et al*, 1956; Truhaut *et al*, 1979). Dead animals showed severe congestion of kidneys, haemoglobinuria, pale liver, and enlarged spleen to concentrated 2-butoxyethanol (0.48 - 0.64 ml/kg bw) whilst surviving animals showed kidney and liver damage and enlarged spleen sinuses. Duprat and Gradiski (1979) found a dermal LD_{50} of 100 mg/kg bw in female rabbits after 8 h of exposure to the undiluted test material under probably occlusive conditions.

In rabbits an LD_{50} of 610 mg/kg bw under occlusive conditions was reported (Roudabush *et al*, 1965).

When 2-butoxyethanol was administered under occlusive conditions for 24 h to the back skin of rabbits (3 animals per dose group; 90.3; 180.6 and 903 mg/kg bw) haemolytic effects were found in the 2 lower groups and lethality and nephroses at the highest level (BASF,1970).

7.2.1.3. Inhalation exposure

In rats (4-5 weeks old) an LC_{50} of 450-490 ppm (males and females) was found after 4 h of exposure. The authors observed loss of co-ordination, red stained urine and enlarged discoloured kidneys in the moribund animals (Dodd *et al*, 1983).

Carpenter *et al* (1956) found an increased osmotic fragility of erythrocytes of female rats at 62 ppm (4 h). The authors also showed an increased susceptibility of older rats; female rats of 250-330 g exposed to 375 ppm for 7 h succumbed, whereas no mortality occurred in rats of 100-120 g (6 weeks old) at 500 ppm (8 h). Necropsy showed enlarged kidneys with tubular necrosis in animals exposed to air concentrations in the LC_{50} range. Increased osmotic fragility of erythrocytes after 4 h inhalation was observable at levels as low as 62 ppm but not at 32 ppm

After a 5 h exposure to 2,400 ppm 8/8 rats had died within 1-2 days post exposure. Comatose state, haematuria and a decrease of haemoglobin (35 - 50% of normal values) were observed (Gage, 1970).

In Swiss mice after 7 h of exposure to 390-1,210 ppm, haemoglobinuria and phagocytic areas in the spleen were observed. The minimum lethal concentration was 700 ppm with a mortality rate of 12.5% (Werner *et al*, 1943a).

In guinea pigs a 7 h LC_{50} was reported to 1,300 ppm (Tyler, 1984).

7.2.1.4. Summary and conclusion

The main cause of death from high doses of 2-butoxyethanol is narcosis. However, there is evidence of kidney toxicity and haemoglobinuria and this appears to be the main cause of death in animals which survive the narcosis. It is likely that the kidney toxicity is secondary to primary haemolysis.

From the limited data available it appears that older animals are more susceptible to these acute effects. Since the metabolism of 2-butoxyethanol is quantitatively different in younger animals, it is likely that this acute toxicity difference is related to the metabolic difference between differently aged animals as well as the increased resistance of the younger red cell to haemolysis.

Haemolytic effects appear fairly soon after exposure. In surviving animals the lysed erythrocytes are replaced by younger reticulocytes which tend to be less susceptible to haemolysis. For that reason the no effect levels in acute studies are similar to the no adverse effect levels in subchronic studies.

7.2.2. Irritation

7.2.2.1. Skin irritation

2-Butoxyethanol as concentrated material under non-occlusive conditions showed only marginal irritative effects in rabbits (Tyler, 1984). Under occlusive conditions (4 h; concentrated material) more pronounced dermal irritation was observed (Rohm & Haas, 1989). Under non occluded conditions the majority of the test material will evaporate (Bartnik *et al*, 1987).

7.2.2.2. Eye irritation

A number of studies have demonstrated that 2-butoxyethanol can act as an eye irritant. In a study to the protocol described in Annex VI to the EC Dangerous Substances Directive, a classification of "irritating" was suggested from the results in rabbits (Jacobs and Martens, 1989).

Other studies in rabbits have also shown marked irritation to the eye from instillation of 2-butoxyethanol (Carpenter *et al*, 1946; Tyler, 1984; Rohm & Haas, 1989).

7.2.3. Sensitisation

2-Butoxyethanol has not shown allergenic effects in a maximisation test in guinea pigs (Unilever, 1989).

7.2.4. Subchronic toxicity

7.2.4.1. General aspects

While some mention of the effect of 2-butoxyethanol on the reproductive organs is made here, for details and a specific discussion see Section 7.2.7.

The primary effects seen in experimental animals following repeated exposure to 2-butoxyethanol are haematological changes (haemolysis, haemoglobinuria) with associated (compensatory) sequelae such as increased proliferative activity in bone marrow and spleen. Secondary toxic changes in liver, kidney and spleen tissue are also frequently reported in animal studies, especially following high dose administration, and may reflect haemosiderosis arising from increased red cell destruction.

7.2.4.2. Oral Administration

In an early study, Carpenter *et al* (1956) administered 2-butoxyethanol in the diet to male and female rats (5 per group) for 90 days at dose levels equivalent to 18, 76, 310 and 1,540 mg/kg bw/d. The actual exposure levels may have been less due to evaporation. The principle findings were an increase in liver weight in the 310 mg/kg bw group, and increased kidney weight in animals from the highest dose group. No haemolytic effects were reported. The value of the study is compromised due to high pneumonia mortality in all groups, including controls.

Grant *et al* (1985) administered 500 or 1,000 mg 2-butoxyethanol/kg bw/d by gavage on four consecutive days to male F344 rats (24 per group). Six animals were subject to haematological examination and necropsy 1, 4, 8 and 22 d after the last treatment. Marked effects on RBC counts and WBC counts were apparent at both treatment levels, with associated splenic extramedullary haematopoiesis, hyperplasia of spleen and bone marrow, and reticulocytosis. The magnitude of these changes was greatest in animals given 1,000 mg/kg bw/d. Apart from a slight residual increase in spleen weight and RBC counts, the effects were fully reversible.

Krasavage (1986) investigated the repeat dose toxicity of 2-butoxyethanol in male CD rats (10 per group) at treatment levels of 0, 222, 443 or 885 mg/kg bw/d, administered 5 d/wk for 6 wk. Two animals from the highest dose group and one from the intermediate group died during the study. Reduced RBC counts occurred throughout the tested dose range, but total

and relative white blood cell counts were unaffected. Red urine (presumably haemoglobinuria) was reported at all doses, but in the lowest treatment group was limited to a single animal on the second day of the study. Kidney and liver changes (haemosiderin accumulation), splenic congestion together with increases in relative liver weight were seen at all dose levels. Relative spleen weight was elevated in the mid- and high dose groups, and increased kidney weight was found in high dose animals. Serum ALP was elevated in the intermediate dose group, and serum ALT increased, and glucose decreased, in the top treatment group. The bone marrow was histologically normal.

The repeat dose toxicity of 2-butoxyethanol after ingestion has been the subject of an extensive investigation by NTP (1993) in rats. In a preliminary investigation, groups of 5 male and 5 female F344/N rats were given 2-butoxyethanol in drinking water for 2 weeks with target doses of 100-650 mg/kg bw/d for 2 wk. There was a dose-related reduction in water consumption which resulted in a below nominal exposure. Females from the top dose group showed a lower terminal body weight and a slight decrease in thymus weight; no gross lesions were observed however. Microscopic investigation was limited to testis and epididymis which showed no treatment-related changes.

In the main investigation (NTP, 1993) rats of the same strain (10 males, 10 females per group) were given 2-butoxyethanol in drinking water for 13 wk at treatment levels of 0, 750, 1,500, 3,000, 4,500, and 6,000 ppm (estimated consumption equivalent to 70 to 500 mg/kg bw/d). All animals survived to the end of the study. A dose-related decrease in terminal body weight was noted for groups exposed to 3,000 ppm and above, which was associated with mild anaemia (decreased RBC count) in males. Thrombocytopenia was present at 4,500 and 6,000 ppm. Treatment-related changes noted at necropsy included reduced thymus weight in males at 4,500 ppm and in both sexes at 6,000 ppm. There was also evidence of slight uterine atrophy but this was considered secondary to the reduction in body weight. Histopathological findings were limited to the liver and haematopoietic system. In the liver, cytoplasmic alterations (eosinophilicity, decreased basophilic granularity), hepatocellular degeneration and deposition of iron in Kupffer cells was commonly observed in treated animals but was more pronounced at exposure levels of 3,000 ppm and above; females seemed especially affected. A haematopoietic response (hyperplasia) was apparent in bone marrow and spleen. There was no treatment-related effect on sperm morphology, and a decrease in sperm concentration, observed at 3,000 ppm and above, appeared to secondary to the decrease in terminal body weight. Results from vaginal cytology indicated that animals from the 4,500 and 6,000 ppm groups had changes in oestrus stages. Stop-exposure studies were also included in 30 male rats per dose group receiving 0, 1,500, 3,000 and 6,000 ppm 2-butoxyethanol via the drinking water for 60 days. At the end of the exposure period animals of the 6,000 ppm group showed lower body weights and decreases of absolute testis weights in all exposure groups. No exposure-related microscopic lesions were observed. The authors concluded that 2-butoxyethanol was relatively non-toxic at the doses tested.

The effects of repeated oral exposure to 2-butoxyethanol have also been studied in mice. Nagano *et al* (1984) reported haemolytic effects in male JCL-ICR mice (5 per group) given

500, 1,000, or 2,000 mg/kg bw/d, 5 d/wk over 5 weeks. The highest treatment level was lethal. Lethality was not observed in a more detailed investigation of the oral subchronic toxicity reported by NTP (1993). In an initial range finding study, groups of male and female B6C3F$_1$ mice (5 per group) were given 2-butoxyethanol in drinking water at levels equivalent to 90 to 1,400 mg/kg bw/d over a period of 2 wk. There was a marginal decrease in final body weight at the highest treatment level, and decreased thymus weights for males from the 370 and 627 mg/kg bw/day groups. There were no treatment-related macroscopic lesions. In the subsequent 13 week study, decreased body weight gain was observed at 3,000, 4,500 and 6,000 ppm. No further treatment-related lesions were detected. Testicular weights and sperm motility were also reduced to an extent which appeared to be secondary to the reduction in body weight gain. The authors concluded that "there were no lesions attributed to 2-butoxyethanol administration in mice" (NTP, 1993).

7.2.4.3. Dermal administration

In a brief report Tyler (1984) indicated that haemoglobinaemia and necrosis at the site of application was observed in female rabbits after nine dermal applications of 180 mg/kg bw (applied as a 50% aqueous solution, 6 h contact period, under occlusion). At 360 mg/kg bw these effects were seen in all animals (both sexes), together with haemoglobinuria and decreased RBC counts. The effects were reversible at the end of a 14 d post-observation period. No adverse effects were seen in animals exposed to 90 mg/kg bw (25% aqueous solution) or below. In another study rabbits were treated daily with an aqueous solution of 2-butoxyethanol (2.8, 14.3, 43.0%, under occlusion) 5 d/wk for 13 wk, with target treatment levels of 10, 50 or 150 mg/kg bw/d (Tyler, 1984; CMA, 1982). No adverse effects were reported.

7.2.4.4. Inhalation exposure

Werner *et al*, (1943b) exposed 23 rats per group to 0, 135 and 320 ppm 2-butoxyethanol for up to 5 wk (7 h/d, 5 d/wk). Animals were killed 1, 3 and 5 wk after the start of exposure, and 1 and 3 wk after treatment ended. There was a transient dose-related fall in RBC count and increased reticulocyte count after exposure week 1, but these parameters gradually returned to normal as the study progressed. Blood haemoglobin values were also decreased and showed a similar time-dependent pattern, although no clear relationship to treatment level was apparent. 2-Butoxyethanol was also found to lyse rat erythrocytes *in vitro*.

Haemoglobinuria was seen in rats (15 per group) exposed to 203, 314 or 432 ppm 2-butoxyethanol for 6 weeks,7 h/d, 5 d/wk, Carpenter *et al* (1956). At the lower dose of 203 ppm, one male and ten females showed signs of haemoglobinuria. All females exposed to 432 ppm died on day 3 of the study, whereas males survived to the end of the study when haemorrhagic lungs and liver enlargement were observed. Exposure levels of 107 ppm and above increased relative liver and kidney weights. Osmotic fragility *in vitro* was noted after exposure to 54 ppm and above.

Gage (1970) found increased erythrocyte fragility in rats (4 per group) exposed to 100 ppm 2-butoxyethanol for 6 h/d on 15 occasions. No effect was seen at 20 or 50 ppm.

A more extensive investigation of the repeat inhalation toxicity of 2-butoxyethanol was reported by Dodd *et al* (1983). In a pilot study, groups of male and female F344 rats (8 per group, age 4 to 5 wk) were exposed to 0, 20, 86 or 245 ppm vapour for 6 h/d on 5 consecutive days and, after 2 d of non-exposure, treatment resumed for a further 4 d (i.e. 9 exposures in total). Haemoglobinuria was noted after the first and second exposure to 245 ppm (both sexes), but not subsequently. Body weights were also decreased in these animals. Dose-related haematological changes (decreased RBC count, decreased haemoglobin concentration, increased reticulocyte count) were observed in both sexes. These parameters showed a substantial recovery during a 14 d post-exposure period. Relative liver weight was increased significantly in the 245 ppm (both sexes) and 86 ppm (females only) groups, but this effect disappeared during the recovery period. No gross lesions were reported as a consequence of exposure to 2-butoxyethanol.

In a subsequent 90 day investigation, with exposure concentrations of 0, 5, 25 and 77 ppm (6 h/d, 5 d/wk for 13 wk) clinical signs of toxicity (including haemoglobinuria) were generally absent and body weight gains comparable to control. The only observation of note was a slight, but significant, decrease in both RBC count and haemoglobin concentration, and increase MCHb in the 77 ppm exposed females after 6 wk. These effects had either lessened or disappeared by the end of the study. No statistically significant effects were observed in serum chemistry or urine analysis values from any of the groups. No treatment-related gross or microscopic lesions were observed (Dodd *et al*, 1983).

In the early investigation conducted by Carpenter *et al*(1956), male mice (70 per group) were exposed to 100, 200 or 400 ppm 2-butoxyethanol vapour 7 h/d for periods up to 18 wk. Increased erythrocyte fragility was seen at all treatment levels, and haemoglobinuria present in the 200 and 400 ppm groups during days 1-3. Increased liver weights were also noted in the mid and high dose groups, although the effect was reversed during a 6 week post-observation period.

Male guinea pigs (10 per group) exposed repeatedly to 54, 107, 203, 376 or 494 ppm 2-butoxyethanol (7 h/d, 5 d/wk) for 6 wk showed evidence of lung and kidney damage at the higher exposure levels but no signs of haemolytic changes. An increase in relative kidney weight was reported at 203 ppm. The NOAEL in this study was 107 ppm (Carpenter *et al*, 1956). Increased kidney weights were also reported in another study in which guinea pigs were exposed to 250 ppm 2-butoxyethanol vapour over a 6 week period. The NOAEL was 125 ppm (Tyler, 1984).

Werner *et al* (1943c) observed focal hepatic necrosis in one of two dogs exposed to 415 ppm 2-butoxyethanol vapour for 12 wk (7 h/d, 5 d/wk). Both animals showed histopathological evidence of chronic inflammatory changes to the lungs and respiratory tract, and an increase (reversible) in blood urea within one week of commencing exposure. No kidney changes, haemosiderosis, extramedullar haematopoiesis, spleen enlargement or haemoglobinuria was

found. In another study (Carpenter *et al*, 1956) two dogs exposed to 415 ppm over 5 wk showed urea retention, presence of calcium oxalate crystals in urine, and a slight reduction in RBC counts, haematocrit value and haemoglobin concentration, but no haemolysis. Increased osmotic fragility, but no discolouration in the urine was reported at 385 ppm in male dog which died after 28 days (a female died on day 8). Exposure to 617 ppm was narcotic, and proved lethal on the second exposure (Carpenter *et al*, 1956).

Transient increases in red cell osmotic fragility, with reduced RBC counts, were reported in two monkeys (male and female) exposed to 100 ppm 2-butoxyethanol vapour for 90 d (Carpenter *et al*, 1956). These changes appeared more pronounced in the female, but had resolved in both animals by the end of the study. Similar effects were also seen in a single monkey exposed to 210 ppm for 30 d but, in this instance, remained until the end of the investigation (Carpenter *et al*, 1956). The effects of 2-butoxyethanol in this study were inconclusive due to the poor health of the experimental subjects.

7.2.4.5. Intraperitoneal injection

Five Sprague Dawley rats received daily injections of 55 mg/kg bw for 2 wk. Five untreated rats served as control. Urinary N-acetyl-ß-glucosaminidase activity, $ß_2$-microglobulin and albumin did not differ from control indicating no toxic effect on the kidney under these conditions (Bernard *et al*, 1989).

7.2.4.6. Summary and conclusion

The subchronic toxicity of 2-butoxyethanol has been extensively investigated in several species using different routes of administration. Mice and rats appear to be the most sensitive species showing increased haemolytic response; this determines the lowest observable effect levels. Nevertheless, in these species, haemolysis is a transient effect predominantly seen in the early stages of studies and the replacing younger RBC and reticulocytes appear to be more resistant to 2-butoxyethanol. The generally recognised inhalation no effect level for haemolytic effects in rats is 25 ppm (6 h/d) in a subchronic study.

Guinea pigs and dogs, in contrast, show little or no effect on erythrocytes as a result of 2-butoxyethanol exposure. For guinea pigs, no effect levels of 107 and 123 ppm have been reported . The observed effects at the next highest exposure level tested (203 and 250 ppm) were lung congestion, and an increase in relative kidney weight in surviving animals, which may have been due to a decreased body weight gain.

It should be noted that all of the inhalation studies reviewed above involved whole body exposure to 2-butoxyethanol vapour. It follows therefore that the no effect levels obtained reflect concurrent dermal and inhalation exposure to 2-butoxyethanol .

7.2.5. Genotoxicity

7.2.5.1. *In vitro*

No mutagenic effects were observed in *Salmonella typhimurium* mutation tests with and without Arochlor induced hamster and rat liver S-9 mix (NTP, 1993). Negative results in *Salmonella typhimurium* were also obtained by Zeiger *et al* (1992).

McGregor (1984) cites three studies by Tyler (1984) which have not been published elsewhere. In a CHO/HGPRT point mutation assay, the cell line was exposed to up-to 1% 2-butoxyethanol in the culture medium for 5 h, with and without rat liver S-9. After a 7 day expression time a single positive response was obtained in the lowest concentration used, but there was no relationship to the dose. The results from this assay were judged to be negative, (McGregor, 1984; UK-HSE, 1984b; NIOSH, 1990).

2-Butoxyethanol did not cause an increase in sister chromatid exchanges in CHO cells exposed to up to 0.25% 2-butoxyethanol in the culture medium for 5 h without an exogenous metabolising system and two hours with rat liver S-9. A repeat test confirmed the lack of response (McGregor, 1984). Induction of sister chromatid exchanges and chromosome aberrations was investigated in CHO cells in the presence and absence of metabolic activation at concentrations up to 5,000 μg/ml. No genotoxic effects were observed (NTP, 1993).

2-Butoxyethanol was examined for its ability to induce unscheduled DNA synthesis in primary rat hepatocytes. Cells were exposed to up to 0.1% 2-butoxyethanol for 2 h in the presence of tritiated thymidine. There was an increase in nuclear and DNA associated radioactivity as measured by liquid scintillation counting but, unusually, only at lower rather than at higher concentrations, (McGregor 1984). This technique is more prone to false positives than direct grain counting.

7.2.5.2. *In vivo*

There are no studies on the *in vivo* genotoxic potential of 2-butoxyethanol.

7.2.5.3. Metabolites

No data are available on the genotoxicity of the metabolites of 2-butoxyethanol.

7.2.5.4. Summary and evaluation

The *in vitro* data show that 2-butoxyethanol has been evaluated in a number of genotoxicity assays and in each case was devoid of significant activity. Whilst there are no *in vivo* data, structural analogues that have been tested *in vivo* show no genotoxic activity. It can be concluded that, like other glycol ethers, 2-butoxyethanol does not have a genotoxic action.

7.2.6. Chronic toxicity and carcinogenicity

No chronic studies have yet been completed. NTP started chronic inhalation studies in rats and mice in July 1993.

7.2.7. Reproductive toxicity

7.2.7.1. Fertility

Male JCL-ICR mice received doses of 500, 1,000 or 2,000 mg/kg bw/d 2-butoxyethanol by gavage for 5 d/wk for 5 wk. The mice were killed on day 6 of week 5 and the effects on the testis and associated structures were observed. Controls received water. At the highest dose levels all the mice died. There were no significant effects on the absolute or relative weights of the testis or seminal vesicles and coagulating gland (Nagano *et al*, 1984). An earlier publication (Nagano *et al*, 1979) mentioned testicular atrophy in one of five mice dosed with 2-butoxyethanol (1,000 mg/kg bw/d). This observation was noted in the early ECETOC publication (ECETOC, 1982), but was not considered significant by Nagano and his co-authors in their later publication (Nagano *et al*, 1984).

Four consecutive administrations of 868 mg 2-butoxyacetic acid/kg bw/d to 6 male SD-rats did not lead to testicular atrophy. In contrast, equimolar doses of methoxy- and ethoxy acetic acid, the metabolites of 2-methoxyethanol and 2-ethoxyethanol, did cause testicular atrophy (Gray *et al*, 1985)

Results from a cross-over mating trial with CD-1 mice receiving 1% 2-butoxyethanol in the drinking water for 19 wk showed some reduction of relative testis weight together with a decrease in male body weights. Sperm concentration, motility and morphology were not altered (Morrisey *et al*, 1988).

In a 2-generation study using the NTP continuous breeding protocol CD-1 mice (20 male and female animals per group) received 2-butoxyethanol via the drinking water. The concentrations were 0, 0.5, 1.0 or 2% (approx. 0; 700; 1,300 and 2,100 mg/kg bw/d) and these were given on the 7 days prior to mating and during the 14 week mating, lactation and gestation period . There was a control group of 40 breeding pairs of mice, (Heindel *et al* 1990). Mortality rates at the respective dose levels were 0/40 (control), 1/20 (0.5%), 6/20 (1.0%) and 13/20 (2%). The material was more toxic to the females. Female partners from 13 and 6 breeding pairs died at the 2 and 1% level respectively. A dose related decrease in water consumption was seen in all treated groups. The body weights of females in high and mid dose groups was reduced and males in the same groups lost bodyweight during treatment. Pathology was done on both the F_o and the F_1 generations. In the F_o generation there was a reduced body weight relative to controls and increased relative kidney weights at 1% and 2%. No effects were seen on the weights of liver, cauda epididymides, epididymides, testes, prostate or seminal vesicles. No effects were seen on the incidence of abnormal sperm, sperm motility or sperm density. Females had decreased bodyweights, increased relative kidney and liver weights relative to controls. No alterations in the oestrous

cycle were found. In the 2% group, 5 of 7 surviving pairs were fertile; but fewer and smaller litters were obtained and the survival rates of the pups were reduced. At 1% all surviving pairs with one exception were fertile. Again, litter sizes, viability rates and pup weights were reduced. A cross mating experiment indicated that reduction in fertility was confined to females, possibly as a secondary effect to systemic toxicity. At 0.5% the body weight of the new-born animals were initially reduced but their fertility was not affected as all animals were fertile . The F_1 generation from the 0.5% dose level showed no effects on the mating and fertility indices, litter size, proportion of live pups or live pup weights. The organ weights were similar to controls except for an increase in liver weights. Again no effects were seen on the incidence of abnormal sperm, sperm motility or sperm density (Heindel *et al,* 1990).

7.2.7.2. Developmental toxicity

7.2.7.2.1. Inhalation studies

In a probe study in rats the exposure levels were 0, 100, 200 and 300 ppm. Reduced food consumption, and weight gain were observed at all levels and reduced red blood cell count at 200 and 300 ppm. At 300 ppm one animal died and 16 of 23 pregnant dams showed 100% resorption. Two foetuses in 2/7 litters at 300 ppm and 1/1 litter at 100 ppm had cardiovascular (ventricular septum) defects. The relevance of these findings as an indication of selective toxicity to the foetus was considered unlikely by the authors (Tyl *et al*, 1984). To investigate these potential effects further, Tyl *et al*, performed a second study in rats and rabbits.

New Zealand rabbits (24 animals per group) were exposed on days 6-18 of gestation to 0, 25, 50, 100 and 200 ppm 2-butoxyethanol for 6 h/d. The animals were killed on the 29th day of gestation. Exposure to 200 ppm caused reduced maternal body weight at day 15. The only treatment related change in maternal organ weights was a reduction in gravid uterine weight, again at 200 ppm. There was evidence of haemolytic effects in the two top dose groups. Corresponding evaluation of reproductive parameters indicated a significant reduction in the number of total implants and viable implants per litter. There was no treatment related effect on the number of non-viable implants, preimplantation loss, percent live foetuses, sex ratio, male and female foetal bodyweight per litter. No treatment related increase in malformations was recorded. The no adverse effect level for dams and foetuses was 100 ppm (Tyl *et al*, 1984).

The same authors exposed Fischer 344 rats (36 animals per group) from days 6-15 of gestation according to the exposure regimen employed in the rabbit study. The animals were killed on the 21st day of gestation. One hundred and 200 ppm caused maternal toxicity as expressed by reduction in bodyweight at 200 ppm and a reduction in body weight gain at 100 ppm. In these two exposure groups an increased number of resorptions, a reduction of living implantations per litter and a retardation of skeletal ossification were noted. No malformations were observed at any dose level. Fifty and 25 ppm showed no adverse effects on dams or foetuses (Tyl *et al*, 1984).

Sprague-Dawley rats (15-16 animals and litters per group) were exposed from days 7-15 of gestation to 150 or 200 ppm for 7 h/d. These levels were selected on the basis of pilot experiments, where 250 and 500 ppm were lethal to the dams. The only adverse effects on the dams was at 200 ppm which led to some haemoglobinuria on the first day of exposure, but not during the subsequent days. No differences in resorption rates or incidence and types of anomalies was found in relation to the control animals. Foetal weights were slightly reduced at 150, but not at 200 ppm. The authors concluded that 2-butoxyethanol was not embryotoxic or teratogenic at the doses tested (Nelson *et al*, 1984).

7.2.7.2.2. Oral screening studies

2-Butoxyethanol was administered in aqueous medium by gavage to Fischer rats from d 9-11 of gestation at dose levels of 0, 30, 100 and 200 mg/kg bw per day or at doses of 0, 30, 100 and 300 mg/kg bw from 11-13 d of gestation. These periods are regarded as the typical intervals for cardiovascular malformations. Thirty mg/kg bw were without effect. At 100 and 200 mg/kg bw haemolytic effects occurred with increased renal and spleen weights. Reductions of body weight gain were observed in the dams. When 200 mg/kg bw were given from day 9 through 11, also an increased foetal lethality without malformations was noted (Sleet *et al*, 1989).

Pregnant CD1 mice were orally administered from day 7-14 post conception 1,180 mg/kg bw/d by gavage (31 animals). 20% of the dams died; the survival rate of the delivered foetuses was 77% versus 100% in the control. No external malformations were seen and no other indication of specific developmental toxicity was found (Schuler *et al*, 1984; Hardin *et al*, 1987).

Wier *et al* (1987) administered 0, 350, 650, 1,000, 1,500 or 2,000 mg/kg bw/d via gavage to pregnant mice (6 animals per group) from gestation days 8 to 14. Haemolytic effects in the dams were observed from 650 mg/kg bw. At 1,500 mg/kg bw the maternal mortality rate was 3/6 and at 2,000 mg/kg bw 6/6. Increased resorption rates and a numerically reduced number of viable foetuses were obtained at 1,000 and 1,500 mg/kg bw. 4/43 foetuses at 1,000 mg/kg bw and 1/25 at 1,500 mg/kg bw had cleft palates. This observation was not confirmed in a subsequent postnatal experiment by the same authors. The dose levels were 650 and 1,500 mg/kg bw per day from gestation days 8 to 14, the litters were raised up to day 22 after birth. No adverse reproductive or developmental effects were observed with this treatment (Wier *et al*, 1987).

7.2.7.2.3. Dermal exposure

Nine Sprague-Dawley rats received daily unoccluded dermal doses of approximately 1,760 mg/kg bw 2-butoxyethanol administered in 4 portions of 0.12 ml (2.5 h intervals) from gestation d 7-16. Nine untreated animals served as control. No maternal toxicity and no indication of malformations, embryotoxicity or foetal resorptions were observed (Hardin *et al*, 1984).

7.2.7.2.4. S.C. injection

Twenty CD rats per group were injected with 0.05, 0.1 and 0.2 ml/kg bw on d 6 through to 15 of gestation. Initial maternal toxicity with haemoglobinuria and weight loss was noted in all dose groups. A slight increase in rib deviations (e.g. supernumerary ribs) and slightly retarded ossification was observed in all treatment groups (Unilever, 1976). In rodents, these findings occur frequently in teratology studies and are commonly interpreted as transient embryotoxicity representing a manifestation of non specific maternal toxicity (Kimmel and Wilson, 1973; Wickramaratne, 1988).

7.2.7.2.5. *In vitro* screening studies

In whole embryo cultures of rats the 2-butoxyethanol metabolite butoxyacetic acid was investigated. No teratogenic effects were observed in contrast to methoxy- and ethoxyacetic acid (Rawlings *et al*, 1985).

Giavini *et al* (1993) found in whole rat embryo cultures an increasing percentage of abnormal development with 2-butoxyethanol and 2-butoxyacetic acid at concentrations of 6.25, 12.5 or 25 mM and 0.8, 1.6 or 3.2 mM respectively. The no effect concentrations were 3.12 mM for 2-butoxyethanol and 0.4 mM for 2-butoxyacetic acid. These results are of little relevance for the *in vivo* situation because such high concentrations of 2-butoxyethanol and its metabolite do not occur in man under normal exposure conditions. Additionally 2-butoxyacetic acid does not accumulate *in vivo*.

7.2.7.3. Summary and Conclusion

2-Butoxyethanol has been well studied for fertility and reproductive effects with a range of well conducted studies using various species, routes and protocols. The weight of evidence shows that 2-butoxyethanol has no effect on male reproductive performance. Whilst 2-butoxyethanol has shown some impact on female fertility this was only at dose levels that caused approximately 30-60% maternal lethality. Doses that were not overtly toxic had no effect upon parental reproductive indices. In prenatal toxicity studies 2-butoxyethanol did not cause teratogenicity. At maternally toxic levels embryo- and foetotoxic effects have been observed. The overall conclusion from all these studies is that 2-butoxyethanol is not selectively toxic to the foetus or reproductive system.

7.2.8. Immunological data

2-Butoxyethanol does not specifically affect the immune system. No selective toxicity was noted in subchronic studies on bone marrow or thymus or white blood cell populations.

In a comparative investigation of various glycol ethers in Fischer 344 rats, 2-butoxyethanol did not suppress the primary plaque forming cell response 3 days following immunisation to trinitrophenyl-lipopolysaccharide (Smialowicz *et al*, 1992).

When the proliferating activity of guinea pig lymphocytes in response to 2-butoxyethanol (2 mM) or butoxyacetic acid (1 mM) was investigated, no cytotoxic effects were noted (Unilever, 1990).

After 21 days exposure via the drinking water (males 2,000 and 6,000 ppm; females 1,600 and 4,800 ppm; 6 animals per sex and dose) an increased natural killer cell (NK) activity was found in each of the lower dose group. The following other immunology parameters were investigated: absolute and relative thymus weight, production of specific antibodies, splenocyte numbers and interferon production in splenocytes. The immune system was not considered as a target organ for 2-butoxyethanol (Exon *et al*, 1991).

In a Fischer rat leukaemia transplant model 8 - 10 animals per group received 3 or 6 mg/ml 2-butoxyethanol in the drinking water for 60 days beginning with a simultaneous s.c. injection of 20 x 10^6 leukaemic cells. Dose-related decreases of RBC indices were noted, but no transplant rejection and no increase of leukaemia latency period (Dieter *et al*, 1990).

7.2.9. Haematological effects

7.2.9.1. General aspects

The early studies of Werner *et al* (1943a, 1943b, 1943c) and Carpenter *et al* (1956) demonstrated that blood was the principle target tissue affected by 2-butoxyethanol *in vivo*. Haemolysis of RBC with associated haemoglobinuria (red staining of urine) was subsequently identified as the primary toxic event following exposure to large doses of 2-butoxyethanol (Note: the term haematuria has been used on occasion to incorrectly describe this observation). These observations have been confirmed in subsequent investigations, and extended to include mechanistic studies in F344 rats. The large number of studies on the haemolytic effects of 2-butoxyethanol in a variety of species is summarised in Table IV.

Table IV
Effects of 2-butoxyethanol on the haematopoietic system of experimental animals

Rat - oral	Dose	Effect
COBS CD(SD)BR, male	222, 443 or 885 mg/kg bw, 5 d/wk for 6 wk	Decreased RBC count and Hb concentration throughout tested range, MCHC reduced in intermediate and high dose groups. Dose-related haemoglobinuria, splenic congestion and renal haemosiderin accumulation noted in all groups. Relative spleen weight increased at 443 mg/kg bw and above. Bone marrow histologically normal (Krasavage, 1986).
F344, male	500 or 1,000 mg/kg bw on 4 occasions	Marked decreases in RBC, HCT, Hb concentration and pronounced increases in MCV, reticulocyte counts and MCHb in some animals at 1,000 mg/kg bw. Changes largely resolved during an 8 d recovery period. Short-lived bone marrow hyperplasia, splenic and hepatic extramedullary haematopoiesis seen microscopically. Changes at 500 mg/kg bw were mild (Grant *et al*, 1985).
F344, male	32-500 mg/kg bw, single dose	Treatment with 125 mg/kg bw or above produced marked haematological effects eg initial increase in HCT, MCV and PCV (at 4 h), followed by destruction of erythrocytes leading to decreased RBC count, HCT and MCHC, with increased MCV and reticulocyte count. Increased relative spleen weight, haemoglobinuria and renal damage (Hb casts, intracytoplasmic Hb) also detected as secondary events (Ghanayem *et al*, 1987b; 1990).
F344, male	125 mg/kg bw, up to 12 daily doses	Time-dependent haemolysis, with reduced RBC counts, Hb concentration and HCT after 1-3 d treatment. Magnitude of haematological effects less marked in animals given 6-12 daily treatments. Relative spleen weight increased to a maximum after 6 daily doses, then declined with subsequent treatments (Ghanayem *et al*, 1992).
RAT-dermal		
SD, pregnant females	0.12 or 0.35 ml applied 4 times daily on d 7-16 of gestation	Red stained urine reported after 1-2 applications of 0.35 ml (maternally toxic). Not seen after treatment with 0.12 ml (Hardin *et al*, 1984).
Wistar, female	200, 260, 375 or 500 mg/kg bw, semi-occluded 6 h contact period	Increased MCV, decreased RBC count, decreased Hb concentration and haemoglobinuria reported at 260 mg/kg bw and above. No effect at 200 mg/kg bw. No clear dose-response relationship observed (Bartnik *et al*, 1987).

Rat - Inhalation	Dose	Effect
Wistar-derived, female	32 or 62 ppm, 4 h exposure	Increased osmotic erythrocyte fragility 62 ppm. No effects at 32 ppm (Carpenter *et al*, 1956).
Wistar-derived, female	200 ppm, 7 h/d on 9 occasions	Increased osmotic erythrocyte fragility after exposure, recovery overnight (Carpenter *et al*, 1956).
Wistar-derived male and female	54, 107, 203, 314, 432 ppm, 7 h/d, 5 d/wk on 30 occasions	Increased osmotic erythrocyte fragility at 54 ppm and above. Haemoglobinuria at 203 ppm and above; greater prevalence in females (Carpenter *et al*, 1956).
F344, male and female	20, 86 or 245 ppm, 6 h/d on 9 occasions	Decreased RBC count, Hb concentration and MCHC and increased MCV on day 8 at highest tested concentration. Magnitude of changes greater in females. Red-stained urine noted after first and second exposure, but not subsequently. Substantial improvement in parameters over a 14 d recovery period. Only mild effects at 86 ppm, normal haematological profile at 20 ppm (Dodd *et al*, 1983).
F344, male and female	5, 25 or 77 ppm, 6 h/d, 5 d/wk for 13 wk	Mild effects (decreased RBC count) after 6 wk or 13 wk exposure to 77 ppm. Hb concentration, HCT, MCV, MCHC, WBC and erythrocyte osmotic fragility unaffected (Dodd *et al*, 1983).
F344, female (pregn.)	50, 100 or 200 ppm, 6 h/d on days 6-15 of pregnancy	Decreased RBC count and increased MCV and MCHb at intermediate and high exposure levels. Animals from high dose group showed additional increase in spleen- and kidney weight. Erythrocyte osmotic fragility unaffected by treatment (Tyl *et al*, 1984).

Mouse - oral	Dose	Effect
JCL-ICR, male	500 or 1,000 mg/kg bw, 5 d/wk for 5 wk	RBC count decreased at both treatment levels. No effect on MCV, Hb concentration or WBC count (Nagano *et al*, 1979).
Inhalation		
C3H, male	100, 200 or 400 ppm, 7 h/d for 30, 60 or 90 d.	Haemoglobinuria after 1-2 treatments and increased osmotic fragility throughout 90 d exposure to 200 or 400 ppm. Haemoglobinuria not seen at 100 ppm, although increased red cell fragility present (Carpenter *et al*, 1956).

Rabbit - dermal	Dose	Effect
New Zealand, male	0.45-0.56 ml/kg bw, 24 h occluded contact	Haemoglobinuria (Carpenter *et al*, 1956).
New Zealand, male and female	18, 90, 180 or 360 mg/kg bw, 6 h occluded contact, 90 applications	Haemoglobinuria in both sexes exposed to 180 or 360 mg/kg bw. Decreased RBC count, Hb concentration and MCHC, and increased MCHb, seen in females at 360 mg/kg bw (Tyler *et al*, 1984).
New Zealand, male and female	10, 50 or 150 mg/kg bw, 6 h occluded contact, 5 d/wk for 13 wk	No adverse effects on haematology or erythrocyte osmotic fragility (Tyler *et al*, 1984).
Rabbit - inhalation		
New Zealand, pregnant female	25, 50, 100 or 200 ppm, 6 h/d on d 6-18 of gestation	No clear evidence of haematoxicity (significant increase in Hb concentration and HCT only at 100 ppm) (Tyl *et al*, 1984).

Guinea Pig- oral	Dose	Effect
strain and sex not reported	250 mg/kg bw (single dose)	No haemolysis. No effect on MCV or HCT (Ward *et al*, 1992).
Inhalation		
strain not reported, male	54-494 ppm, 7 h/d, 5 d/wk for 6 wk	No haemoglobinuria. No effect on erythrocyte osmotic fragility (Carpenter *et al*, 1956).

Dog - inhalation	Dose	Effect
Basenji, one male, one female	200 ppm, 7 h/d for 31 d	Slight increase in red cell osmotic fragility (both sexes), with slight reduction in RBC count and Hb concentration in female only. WBC count doubled in male (Carpenter *et al*, 1956).
Basenji, single male	385 ppm, 7 h/d, 28 d	Increased erythrocyte osmotic fragility (maximal at day 7). Animal died on day 28 (Carpenter *et al*, 1956).
Wire haired terrier, one male, one female	100 ppm, 7 h/d for 90 d	Decreased haematocrit at study end. WBC count increased two-fold at midpoint of investigation (Carpenter *et al*, 1956).

Monkey - Inhalation	Dose	Effect
Rhesus monkey, sex not specified	210 ppm for 30 d (period of exposure not specified)	Increased erythrocyte osmotic fragility noted after fourth exposure, present throughout remainder of study. RBC count and Hb concentration decreased by approx. 50% at study end (Carpenter *et al*, 1956).
Strain not specified, one male, one female	100 ppm, 7 h/d for 90 d	Occasional increases in red cell osmotic fragility (more pronounced in female), transient mid-study decrease in RBC count (Carpenter *et al*, 1956).

7.2.9.2. Mechanisms of haemolysis *in vivo*

A detailed haematological investigation into the effects of repeated short term oral administration of large dose of 2-butoxyethanol was conducted by Grant *et al* (1985). Male F344 rats (4-5 wk) were given doses of either 500 or 1,000 mg/kg bw/d for 4 consecutive days. Blood samples were taken from 1 to 22 days after the last treatment. The characteristic effects on erythrocyte numbers and increased relative spleen weight reported by Ghanayem *et al* (1992) were seen at both doses, although at 500 mg/kg bw these blood changes were relatively mild. Most of these parameters had returned to normal 22 d after treatment, at which point the study was terminated. As well as describing the standard haematological findings, the report noted that the treated rats showed increased numbers of circulating nucleated RBCs; these changes had generally resolved by day 8. Leukocyte counts were decreased 24 h after the end of the high dose treatment due, principally, to a reduced number of circulating lymphocytes. Microscopic examinations revealed short-lived bone marrow hyperplasia and splenic and hepatic extramedullary haematopoiesis and lymphocyte depletion of the thymic cortex.

Marked haematological effects, together with increased relative spleen weights, were seen when adult (9-13 wk) male F344 rats were given a single dose of 125-500 mg/kg bw by gavage. There were initial time- and dose-dependent increases in HCT, MCV and PCV (thought to be due to a spherical swelling of the erythrocytes), followed by the destruction of RBCs. This resulted in time- and dose-dependent decreases in circulating RBCs, HCT and MCHC, along with an increase in free Hb in the plasma, and increased MCV and reticulocyte count. Most of these changes were detectable within 2 h of treatment, although the effects on reticulocyte count were not seen until 48 h after dosing. This increase in reticulocyte numbers was thought to represent an adaptive response by bone marrow to the reduction in circulating erythrocytes. Secondary to the haemolytic action were an increase in relative spleen weight, haemoglobinuria and kidney damage (Hb casts and intracytoplasmic Hb). There were also clearly linked histopathological changes seen in liver tissue (i.e. phagocytosed haemoglobin in hepatic parenchymal- and Kupffer cells); focal hepatic necrosis was also detected at 250 and 500 mg/kg bw, but it was unclear if this was directly related to the haematological effects (Ghanayem *et al*, 1987b; 1990)

The sensitivity of F344 rats to the haematological effects of 2-butoxyethanol showed a clear dose- and age-dependence, as demonstrated by Ghanayem *et al* (1987b). A significant decline in RBC counts, Hb concentration and HCT was detected in 9-13 wk old rats after gavage administration of 125 mg/kg bw 2-butoxyethanol, but this treatment was without effect in 4-5 wk old animals. The magnitude of these changes increased with dose, but young rats appeared to be less susceptible than adults. Furthermore, the kidney and liver pathology seen in the adult animals at the higher doses was not found in the 4-5 wk old animals. These findings were considered by the investigators to be a direct consequence of the greater formation of 2-butoxyacetic acid in the older animals (see section 7.1.4).

Additional support for the hypothesis that 2-butoxyacetic acid was involved in 2-butoxyethanol-induced haematotoxicity was obtained from inhibitor studies with pyrazole, an inhibitor of ADH and cyanamide, an inhibitor of ALDH activity (Ghanayem *et al*, 1987c). In these investigations, F344 rats given a single oral treatment of 2-butoxyethanol (125 mg/kg bw) showed typical changes in spleen weight and haematological parameters (haemolysis, reductions in RBC counts, Hb concentration and HCT), but these were almost abolished in animals pre-treated with pyrazole; reduced conversion of 2-butoxyethanol to 2-butoxyacetic acid was thought to be the explanation. Similarly, administration of 2-butoxyacetaldehyde (125 mg/kg bw) caused damage to the spleen and erythrocytes, but these effects were decreased in rats pre-treated with cyanamide. In this instance, reduced conversion of 2-butoxyaldehyde to 2-butoxyacetic acid was the mechanism proposed.

Tolerance to the haematological properties of 2-butoxyethanol has been reported in several of the repeat dose studies reviewed in section 7.2.4. This phenomenon was investigated in more detail in adult (10-14 wk) male F344 rats by Ghanayem *et al* (1992). The results showed that RBC counts were decreased by approximately 50% (47-57% of control) after 1-3 daily treatments with 125 mg/kg bw 2-butoxyethanol. In contrast, decreases of approximately 30% were seen following 6-12 treatments (RBC count 66-76% of control). By the end of the study (i.e. after 12 doses), RBC counts were approaching pretreatment values. Relative spleen weight and reticulocyte count were also increased up to day 6, then declined. Animals which had younger population of RBCs, as occurred after bleeding or after haemolytic doses of 2-butoxyethanol, were considerably less sensitive to the haemolytic action of a subsequent dose of 2-butoxyethanol.

7.2.9.3. Haemolysis *in vitro* and species differences

High concentrations of 2-butoxyethanol can lyse erythrocytes of rats and man *in vitro*, probably mediated by a solvent effect. The metabolite 2-butoxyacetic acid lyses erythrocytes from rats, but not man, at far lower dose levels (concentration x time). Haemolysis by 2-butoxyethanol or 2-butoxyacetic acid *in vivo* is preceded by cell swelling (spherocytosis) and ATP reduction. This, however, is not observed with 2-butoxyethanol *in vitro*. In contrast to the 2-butoxyethanol solvent effect, the lysis mediated by 2-butoxyacetic acid may follow a different mechanism. Ghanayem (1989a) hypothesised that butoxyacetic acid may disturb the osmotic balance of the erythrocyte plasma membrane.

Carpenter *et al* (1956) showed that human erythrocytes *in vitro* are much less susceptible than rat erythrocytes to 2-butoxyethanol (2.5%) and 2-butoxyacetic acid (0.1%) and need a longer time to show lytic effects.

In the experiments of Bartnik *et al* (1987) rat erythrocytes showed 100% haemolysis after 2 h incubation with 175 mmol/l 2-butoxyethanol, whereas the response in human erythrocytes was 23% haemolysis under these same conditions. At 200 mmol/l rat erythrocytes were 100% haemolysed after 30 minutes, whereas human erythrocytes showed a 100% haemolysis only after 2 h. When incubated with 2-butoxyacetic acid (7.5 mmol/l) complete haemolysis of rat erythrocytes occurred within 1 hour, whereas no effect was observed in human erythrocytes incubated with up to 15 mmol/l for 3 h.

Ghanayhem *et al* (1989a,b) incubated rat erythrocytes with 2 mmol/l 2-butoxyacetic acid and found a near-complete haemolysis after 4 hours. In human erythrocytes exposed to 0.5, 1.0, 2.0, 4.0 and 8.0 mmol/l 2-butoxyacetic acid, only minor haemolysis was found at 8.0 mmol/l, 4.0 mmol/l was a no effect concentration. RBCs from females appeared to be slightly more sensitive.

Other studies have confirmed species differences in susceptibility to 2-butoxyacetic acid induced haemolysis *in vitro*. For example, RBCs from rats, mice, rabbits and baboons are sensitive to this effect, whereas red cells from pigs, cats, dogs, guinea pigs and humans are less sensitive or resistant (Ward *et al*, 1992; Ghanayem and Sullivan, 1993). Furthermore, no human population hypersusceptible to 2-butoxyacetic acid have been identified in studies using RBCs from young or old and male or female subjects (Udden, 1994). Even RBCs from human patients with established sickle cell disease and hereditary spherocytosis (a clinical condition resulting in a predisposition to haemolysis) did not show an haemolytic effect after incubation with butoxyacetic acid *in vitro* at concentrations (2 mM) which caused haemolysis in rat RBCs (Udden, 1994).

7.2.9.4. Summary and conclusions

The haemolytic potential of 2-butoxyethanol has only been studied in any detail in F344 rats. In this strain, oral or inhalation exposure results in marked reduction in the number of RBCs in the peripheral circulation. The haemolytic effect is more marked in older than in younger rats, and after shorter rather than longer exposure. Although age may also affect the pharmacokinetics of 2-butoxyethanol in old and young rats (section 7.1.4), the lower sensitivity of the younger animals is also due to the higher proportion of young erythrocytes and reticulocytes present in the circulation. The tolerance observed on repeated exposure is thought to have a similar basis, the early exposure preferentially destroying the older erythrocytes. The younger red cells that replace them appear to be relatively resistant to the haemolytic action of 2-butoxyacetic acid. There is some weak support for the view that female rats are more susceptible than males to the haemolytic action of 2-butoxyethanol.

Generally similar blood effects have been seen in other strains of rat as well as other species of animal (mouse, rabbit, baboon). These changes are observed following oral, dermal or

inhalation exposure. In contrast, there is information *in vivo* to suggest that the cat, dog, guinea pig and humans are relatively resistant to this haemolytic action. This is supported by *in vitro* data (see section 7.2.10.2).

7.2.10. *In vitro* cytotoxicity assessments

In haemopoietic cell lines (either leukaemic or growth dependent) the median inhibitory concentration of several glycol ethers (IC_{50}) was investigated. In human promyelocytic cell line NB4, 2-butoxyethanol was found to exert toxicity at 5 mM after 6 h and at 0.1 mM after 96 h. In the factor-dependent cell line DA1 an IC_{50} value of 80 mM (48 h) was found. The dose response relation (all or none toxicity) appeared to be steep in a narrow concentration range (Ruchaud *et al*, 1992).

2-Butoxyethanol is not metabolised in most established cell lines due to their inadequate competence for xenobiotic metabolism. Therefore, the results of investigations in cell lines do not necessarily mirror the *in vivo* activities of 2-butoxyethanol. Hence, *in vitro* investigations on 2-butoxyethanol related toxicity usually use 2-butoxyacetic acid (Rawlings *et al*, 1985; Jäckh *et al*, 1985; Bartnik *et al*, 1987; Foster *et al*, 1987; Ward *et al*, 1992; Udden and Patton, 1994; Udden, 1994).

In a colony formation cytotoxicity assay in CHO cells, 2-butoxyethanol showed an IC50 of 0.05 mmol/ml (6.9 mg/ml; 16 h exposure), and was about 10 times more toxic than methoxyethanol. However, butoxyacetic acid showed an IC_{50} of 0.04 mmol/ml (16 h), which was nearly the same as for methoxyacetic acid with 0.05 mmol/ml (Jäckh *et al*, 1985). It is clear that the profound differences in toxicity to developing tissues *in vivo* is not reflected by such *in vitro* techniques in established cell lines.

7.3. EFFECTS ON HUMANS

7.3.1. Case reports

Limited information on the effects of 2-butoxyethanol ingestion by man is available following deliberate (suicide) or accidental consumption.

Two cases of severe poisoning after suicide attempts with hard surface cleaning formulations were reported by Rambourg-Schepens *et al* (1988) and Gijsenbergh *et al* (1989). Both cases were treated successfully.

In the first case 250-500 ml of a formulation containing 12% 2-butoxyethanol were ingested by a 50 year-old woman. She was admitted to hospital 12 h later in deep coma. Moderate haemoglobinaemia and metabolic acidosis were the major symptoms of toxicity seen. There was a concomitant and progressive fall in RBC count, oxaluria, metabolic acidosis, hypocalcemia. An increase in serum creatinine level was also observed. The symptoms developed on the third day and lasted for 3 days, and were associated with a progressive

erythropenia. A reticulocyte count was not made. The patient was discharged from hospital ten days after the incident occurred.

In the second case a 23 year-old woman ingested approximately 500 ml of a window cleaner containing 2-butoxyethanol and ethanol. She was admitted to the hospital 1 hour later in a comatose state and showed severe metabolic acidosis. From blood ethanol measurements it was estimated that approx. 200-250 ml of cleaner could have been absorbed, corresponding to 25-30 g 2-butoxyethanol. "Routine blood laboratory examination" on admission to hospital about 1 h after the incident revealed no abnormalities. Blood Hb, which was 11.9 g/dl on arrival at the hospital, had fallen to 8.9 g/dl on the second day, and was associated with haematuria. The patient recovered after forced diuresis and haemodialysis, and was discharged from hospital after 8 days (Gijsenbergh *et al*, 1989).

Hypochromic anaemia (Hb 9.1 g/dl; haematocrit 25%) with thrombocytopenia (platelet count 85,000) was reported in a male alcoholic 48 h after ingestion of 500 ml of a cleaning fluid containing 9.1% 2-butoxyethanol (equivalent to the ingestion of around 45g) and 25% ethanol. The patient was discharged after 15 days, following supportive treatment and haemodialysis. The anaemia was described as "non-haemolytic", but no support was offered for this diagnosis (Bauer *et al*, 1992).

Browning (1965) reported a case from 1934 in Britain where a worker experienced 2 isolated attacks of haemoglobinuria. The attacks were within 5 months of each other and occurred after occupational overexposure to a mixture of 2-butoxyethanol and diethylglycolmonobutyl ether (Note: the details of this study are inadequate to ascribe the findings to 2-butoxyethanol).

Finally, in a survey of paediatric poisonings reported to the Pittsburgh Poison Center over a 5 month period, a total of 24 children were identified who had ingested 5-300 ml of domestic products containing 2-butoxyethanol. The two highest intakes involved the consumption of approximately 3 ml or 24 ml 2-butoxyethanol (no details were provided on bw), but no evidence of haemolysis or other effects were reported in either case (Dean and Krenzelok, 1992).

7.3.2. Controlled studies

Carpenter *et al* (1956) carried out controlled investigations in human volunteers in 3 sequential experiments. In the first study 2 men were exposed simultaneously with 6 rats to 113 ppm for 4 h. The participants showed irritation of nose, eyes and throat. In a later experiment study the same 2 men and 1 woman were exposed to 195 ppm for two 4-h periods separated by a 30 minutes break. Subsequently, the woman suffered from nausea and headache for 24 h. In a third experiment, 2 men and 2 woman were similarly exposed to 100 ppm for 8 h. Again discomfort, nausea and headache were experienced. From these experiments it was clear that exposure conditions of 100 ppm and more could not be tolerated by man. No clinical signs of haemolysis were observed, but blood samples of exposed individuals showed an increased osmotic erythrocyte fragility after exposures to

195 ppm (but not at 100 ppm). Rats co-exposed in the first study to 113 ppm showed evidence of haemolysis.

2-Butoxyacetic acid levels were measured in urine collected from the volunteers 24 h post exposure. One of the two men from the second experiment (195 ppm) had an excretion rate of 175 mg butoxyacetic acid/24 h, whereas in the urine of the other man only trace amounts of butoxyacetic acid were detected. (This man was also in the third experiment where he showed a similar excretion rate as the others). An excretion rate of 300 mg butoxyacetic acid/24 h (following exposure) was observed in the woman at 195 ppm. She also had the severest symptoms. The other urinary butoxyacetic acid excretions at 100 ppm were in the range of 75-250 mg/24 h showing that inter-individual variabilities in 2-butoxyacetic acid excretion are very wide for a given exposure.

Johanson *et al* (1986a) investigated 2-butoxyethanol uptake and metabolism in seven men during light exercise and exposed for 2 h to the Swedish occupational inhalation standard of 20 ppm. In a later study (Johanson *et al*, 1988) the dermal uptake in 5 male volunteers immersing 4 fingers for 4 h into undiluted 2-butoxyethanol was investigated. Johanson and Boman (1991) exposed volunteers dermally to 2-butoxyethanol vapours of 50 ppm under respiratory protection; dermal absorption was observed under conditions where the exposed persons only wore shorts. The same individuals were exposed to 50 ppm 2-butoxyethanol for 2 h via a face mask. No signs of respiratory irritation or systemic toxicity were reported. (See section 7.1.2 for further details of these studies).

2-Butoxyethanol, as a 10% aqueous solution, has been evaluated in a human repeat insult patch test for skin sensitization. There was no evidence for skin sensitization in any of the 200 individuals tested (CMA, 1992).

7.3.3. Occupational studies

Vincent *et al* (1993) determined the exposure levels of 16 office cleaning women and 13 garage car cleaners (11 men, 2 women) who used 2-butoxyethanol based window cleaning agents. Monitoring was by personal air sampling and by determining 2-butoxyacetic acid levels in pre- and end of shift urine. The time weighted average levels of 2-butoxyethanol were up to 7.33 ppm at maximum (< 0.10 - 7.33 ppm) and the 2-butoxyacetic acid levels in urine < 2 - 371 mg/g creatinine in end of shift samples. In the higher exposed groups (mean 2-butoxyethanol level 2.44 ppm), 111.3 mg/g creatinine were found. A near linear relation was observed between the amount of window cleaning agent (0.9 - 22.1% 2-butoxyethanol content v/v) used and the mean 2-butoxyacetic acid excretion, whereas no clear correlation appeared for the 2-butoxyethanol air concentrations and mean 2-butoxyacetic acid excretion. On the other hand, the highest 2-butoxyethanol levels (2.85 - 7.33 ppm) were found in the same garage where most of the 2-butoxyethanol (in daily quantity) was used. The preshift levels were approximately a factor of 2 - 10 (mostly 4) lower than end shift levels. The garage workers used 50 - 900 ml of cleaning agents over 120 - 300 minutes/day. Among office cleaners urinary 2-butoxyacetic acid was only detected in 3/32 samples (4.6 mg/g creatine at maximum) and only in the group where 2-butoxyethanol levels exceeded 0.3 ppm.

However, the office cleaners used less than 50 ml of cleaning agent containing 2-butoxyethanol in a day and its use was usually spread over as little as a 15 minute time frame. From these results the authors concluded that skin absorption from liquid formulation was the predominant exposure route. No clinical signs of toxicity or intolerance were noted in this study.

In an unpublished study, Van Vlem (1987) examined the relationship between 2-butoxyethanol exposure and 2-butoxyacetic acid excretion in the group of 5 women investigated by Veulemans (1987) at a silk screening operation. The mean inhalation exposure concentrations were 0.65 ppm (3.1 mg/m^3). In all cases higher 2-butoxyacetic acid concentrations in urine were found post shift (8 - 11 mg/l) than preshift (1.0 - 1.5 mg/l). After the weekend no 2-butoxyacetic acid was detected. The calculated half life of 2-butoxyethanol was 8.3 h. This showed that no accumulation occurred during the workweek.

7.3.4. Biological monitoring

Following the first reports of Carpenter *et al* (1956) and the more comprehensive studies of Johanson (1988), the concentration and excretion rate of 2-butoxyacetic acid would be expected to reflect exposure to 2-butoxyethanol via all routes of exposure. The material is not present in unexposed persons. A refined analytical method has been recently recommended and described by Rettenmeier *et al* (1993). 2-Butoxyacetic acid appears to exert the major part of 2-butoxyethanol related toxicity. Experimental signs and subjective symptoms may correlate with 2-butoxyacetic acid excretion. However, the correlation between external 2-butoxyethanol exposure and 2-butoxyacetic acid excretion are less established and may show considerable inter individual variability e.g. in uptake and in ADH mediated butoxyacetic acid production. For that reason, Johanson *et al* (1988) recommended the collection of several urine specimens. Two mg/l appears to be the detection limit (Vincent *et al*, 1993).

Instead of collecting the 24 h urine, 2-butoxyacetic acid excretion may also be estimated from a single probe corrected for the creatinine level. In 1990, NIOSH assumed at end shift after an 8 h workday under an airborne level of 5 ppm 2-butoxyethanol, 60 mg butoxyacetic acid/g creatinine as an excretion value may be expected. In contrast Vincent *et al* (1993) reported mean end shift excretion of up to 111 mg 2-butoxyacetic acid/g creatinine following exposure to a mean exposure of 2.3 ppm 2-butoxyethanol but there was a dermal component to the systemic dose.

Rettenmeier *et al* (1993) reports the glutamine conjugate of butoxyacetic acid as a significant urinary metabolite (up to 30% of the total 2-butoxyacetic acid excretion). Current analytical methods may not detect this metabolite.

8. GAPS IN KNOWLEDGE AND ONGOING RESEARCH

8.1. HAEMATOLOGICAL EFFECTS

To further the understanding of the difference between rats and humans to 2-butoxyacetic acid-induced haemolysis, studies to determine the mechanism(s) for susceptibility of rat RBCs, and the apparent resistance of human cells to haemolysis by 2-butoxyacetic acid should be considered. It is believed that such studies might reveal fundamental differences between rat and human RBCs which would indicate why human beings are apparently not susceptible to haemolysis by exposure to 2-butoxyethanol, whereas rats and several other species are.

Additional information on the effects of 2-butoxyethanol in a non-haemolytic species would be valuable in hazard assessment.

A physiologically-based pharmacokinetic model for 2-butoxyethanol and 2-butoxyacetic acid was developed for the rat and extended to man (Corley *et al,* 1993). Although the model currently predicts 2-butoxyacetic acid blood levels and excretion rates for rats under oral, dermal, inhalation and intravenous routes of exposure, further studies are currently underway to refine the model.

8.2. ABSORPTION AND PHARMACOKINETICS

CMA is currently sponsoring additional studies to address the dermal bioavailability of 2-butoxyethanol vapours in humans. Previously, Johanson and Boman (1991) had sampled finger-prick blood from human beings exposed dermally or by inhalation to 2-butoxyethanol, and assumed these were representative of systemic blood, rather than blood draining from the dermal site of absorption. The authors concluded that the dermal route was quantitatively more significant than inhalation. In the CMA studies, humans will have one arm exposed in a chamber to 50 ppm 2-butoxyethanol vapours for up to 4 hours. Blood samples will be withdrawn by finger-prick both from the exposed arm (as for Johanson and Boman, 1991), and directly from the vein of the unexposed arm and analysed for 2-butoxyethanol and 2-butoxyacetic acid. A comparison of levels in the two blood samples will determine if finger-prick blood is a surrogate for systemic blood. The levels of 2-butoxyethanol and 2-butoxyacetic acid in the venous blood will be useful in the PBPK model to assess the rate and extent of dermal penetration, and the relative importance of dermal versus inhalation bioavailability of vapours of 2-butoxyethanol.

8.3. GENOTOXICITY

The French National Research and Safety Institute (INRS, 1993) has an ongoing research project to further examine the genotoxic effects of glycol ethers including 2-butoxyethanol *in vitro* and *in vivo*.

8.4. CARCINOGENICITY

NTP has done prechronic inhalation studies in rats and mice for the purpose of dose-setting for a two year bioassay. The oncogenicity studies started in July 1993. Fischer 344 rats are being exposed whole body to 31, 62.5 or 125 ppm, and B6C3F1 mice to 62.5, 125, or 250 ppm, 6 h/d, 5 d/wk. As well as the standard oncogenicity bioassays, additional studies include limited toxicokinetics, bone marrow cytology and stem cell proliferation assays.

A concern with performing any studies, including oncogenicity studies, with 2-butoxyethanol in a species which is susceptible to haemolysis is that any toxic effects may be secondary to intravascular haemolysis. This may compromise their use for hazard assessment in man, a species which appears to be resistant to haemolysis under realistic exposure conditions.

8.5. EPIDEMIOLOGY

The French National Research and Safety Institute (INRS, 1993) plans to gather data on any haematological cases known to have occurred in workplaces using glycol ethers including 2-butoxyethanol.

They also propose a case-controlled epidemiological study of toxic effects of exposure to ethylene glycol ethers.

9. GROUPS AT EXTRA RISK

No groups at extra risk or specifically susceptible to 2-butoxyethanol have been identified. Results from a limited number of exposed volunteers (Carpenter *et al*, 1956) showed a single woman to be more affected than the men in the same study.

Studies in young and old rats indicate that the internal dose of 2-butoxyacetic acid and the susceptibility to haemolytic effects may increase with age. However, *in vitro* studies have shown that human erythrocytes from older individuals are not more susceptible to haemolysis than RBC from young individuals. Human subpopulations with inborn genetic disorders predisposing to haemolytic diseases are also not more susceptible than the average population; this has been demonstrated by recent *in vitro* investigations (Udden, 1994).

2-Butoxyethanol vapours may be absorbed to some extent through the skin. This absorption may be increased with moisturizing of the dermis. For this reason high air humidity and heavy labour may increase the internal dose levels.

10. REVIEW OF EXISTING ASSESSMENTS

Two inhalation exposure standards for 2-butoxyethanol have been proposed in the United States, one by the American Conference of Governmental Industrial Hygienists (ACGIH, 1991) and another by the National Institute of Occupational Safety and Health (NIOSH, 1990). Both are based on animal studies without consideration of recent human studies.

The ACGIH TLV-TWA for 2-butoxyethanol is 25 ppm (ACGIH, 1991). An animal no-observed-effect-level (NOEL) of 25 ppm was taken from the Dodd *et al* (1983) rat subchronic inhalation study. At the next highest concentration of 77 ppm there were slight haematological effects. ACGIH recognised that the rat was more sensitive than humans to the haemolytic effects of 2-butoxyethanol, but realised it would be unusual to set a human occupational standard at a higher concentration than an animal NOEL. ACGIH also recognised that at concentrations much above 25 ppm, upper respiratory tract irritation would become a factor.

Using a different approach, NIOSH (1990) proposed a Recommended Exposure Level (REL) of 5 ppm for the workplace. NIOSH chose to use a rat inhalation developmental toxicity study (Tyl *et al*, 1984) where the NOEL was reported to be 50 ppm. No developmental toxicity was reported rather, haemolysis was the toxic effect, as for the subchronic study in rat (Dodd *et al*, 1983) . Assuming 100% retention of inhaled 2-butoxyethanol, a rat body weight of 0.215 kg, and a rat inhalation rate of 0.161 m^3/d and 6 h/d exposure, NIOSH calculated a daily systemic dose for the rat of 44.9 mg/kg bw. The reverse calculation was performed from a human systemic dose of 44.9 mg/kg bw, again assuming 100% absorption, and 70 kg bw and 10 m^3 air is inhaled per 8 h work day. The resulting equivalent human exposure was 314 mg/m^3, or 65 ppm. At this point NIOSH applied an intraspecies variability (sensitive individual) uncertainty factor of 10, to arrive at a REL of 6.5 ppm, rounded down to 5 ppm.

NIOSH recognised that no interspecies uncertainty factor was necessary, because the equivalent doses for rat and man had been corrected by physiological considerations; also, no interspecies (rat to man) uncertainty factor was necessary because the rat was more sensitive than man, based on limited data at that time. However, NIOSH did not apply a fractional safety factor to quantify the decreased sensitivity of human beings compared to the animal model.

The German MAK-Commission proposed an 8 h TWA of 20 ppm (DFG, 1983) based also on an animal NOEL of 25 ppm as reported in the rat study of Dodd *et al* (1983). No uncertainty factors were applied, since it was recognised that in terms of haemolysis man is less sensitive than rat. A skin notation was considered necessary.

11. EXISTING OCCUPATIONAL EXPOSURE LIMITS

A variety of occupational exposure limits are in operation within the EC and in other countries. Details are give in the table below (Table V).

Table V
Occupational Exposure Limits for 2- Butoxyethanol in various countries

Country	Standard name	Time (TWA)	Value	Notes / Comment
Austria	MAK	8 h	20ppm (100mg/m^3)	MAK Peak Limitation Category II,1 (systemic effects)
France	VLE	8 h	25ppm	Considered to be a danger of cutaneous penetration
Germany	MAK	8 h	20ppm	MAK Peak Limitation Category II,1 (systemic effects) MAK Skin Indicator: H (Cutaneous Absorption) MAK Pregnancy Group : C (No reason to fear risk of damage) DFG (1983)
Holland	MAC (TWA) MAC (STEL)	8 h 15 minutes	20ppm 40ppm	Note - Danger of Cutaneous absorption
Norway	TLV	8 h	20ppm	
Sweden	Level Limit Value (NGV) Short term Limit (KTV)	8 h	20ppm	Notes - The substance can easily be absorbed through the skin
Switzerland	TLV	8 h	20ppm	
UK	Maximum exposure limit	8 h	25ppm	Ceiling Limit value Skin notation Comment - based on reproductive effects as reviewed in HSE (1984b)
UK	Short term exposure limit	15 minutes	50ppm	
USA - ACGIH	Threshold Limit Value	8 h	25ppm	Skin notation
USA - NIOSH		8 h	5ppm	Recommended exposure level
USA - OSHA	Permissible Exposure Limit	8 h	50ppm	OSHA final rule limit applies to skin. (Previous value of 25ppm revoked - June 1992)

12. SUMMARY EVALUATION AND RECOMMENDATION FOR A SCIENTIFICALLY BASED OCCUPATIONAL EXPOSURE LIMIT

12.1. SUBSTANCE IDENTIFICATION, CHEMICAL AND PHYSICAL PROPERTIES

12.1.1 Identification

Common Names	2-Butoxyethanol Ethylene Glycol (mono) n-Butyl Ether (EGBE)
Chemical Abstracts Registry No.	111-76-2
EEC No	603-014-00-0
EEC Classification	Xn (Harmful) R20/21/22 Xi (Irritant) R37
EEC Labelling	Symbol Xn Risk Phrases 20/21/22-37 Safety Phrases 24/25
EINECS No.	203-905-0
Formulae	$C_6H_{14}O_2$
Structure	$CH_3CH_2CH_2CH_2OCH_2CH_2OH$

12.1.2. Chemical and physical properties

2-Butoxyethanol is a colourless liquid with a mild ether odour. It is soluble in water and in most organic solvents. Major properties are given in the table below (Table VI).

Table VI

Property	Value[1]
Molecular weight	118.2
Molecular formula	$C_6H_{14}O_2$
Specific gravity at 25°/4°C	0.898
Evaporation rate (butyl acetate = 1.00)	0.1
Boiling point °C	170.8
Freezing Point °C	-77
Vapour pressure (hPa, at 25°C)	1.17
Refractive index	1.417
Flash point (°C), closed cup	62
Autoignition temperature (°C)	238
Flammability limits (volume % in air)	1.10 - 12.7
Water solubility	Soluble
Vapour density (air = l)	4.1
ppm in saturated air (25°C)	1,200
Surface tension (mN/m at 25°C)	27.4
Conversion factors 1 ppm = 4.91 mg/m^3 (20 °C, 114 hPa) 1 mg/m^3 = 0.204 ppm (20°C, 114 hPa)	

1. Rowe and Wolf (1982)

12.2. PRODUCTION, USE AND EXPOSURE LEVELS

12.2.1. Production and use

2-Butoxyethanol is the most widely produced glycol ether with a production capacity in the European Community of approximately 70,000 tonnes per year . It is usually synthesised by a reaction of ethylene oxide with butyl alcohol. Ethylene glycol monoalkyl ethers are not formed as pure compounds but must be separated from the monoethers of diethylene glycol,

triethylene glycol, and the higher glycols. Temperature, pressure, reactant molar ratios, and catalysts are selected to give the product mix desired.

In most applications 2-butoxyethanol is used because it is an excellent coupling agent. It is a small yet vital component of many water born coating and cleaning technologies. It is used in the formulation of many industrial and consumer products such as inks, coatings, cleansers, and fuel additives and as a solvent in surface coatings such as paints, spray lacquers, quick-dry lacquers, enamels and varnish removers. 2-Butoxyethanol is employed as an intermediate in 2-butoxyethanol acetate production.

12.2.2. Exposure levels at the workplace

Exposure to 2-butoxyethanol occurs during production and during the end use of formulated products. During production there are very few people exposed to 2-butoxyethanol. Typically there are no personnel working constantly on the plant, only occasional visits from fitters, engineers and other technical staff. There is the potential for exposure to the chemical to personnel in control rooms, but again this is minimal (2 to 4 people per shift per production facility) There are several plants producing 2-butoxyethanol in the European Community.

There are a few reports of quantitative workplace (non production area) exposure studies. Recently, a biological exposure index for 2-butoxyethanol has been developed, and used to determine the systemic dose after exposure to formulated products by automobile- and window cleaners (Vincent *et al*, 1993). The formulated cleaning products contained 2-butoxyethanol at concentrations ranging from 1-21%. The results indicated that inhalation exposure was a minor component of the systemic dose (highest mean air concentration about 5 ppm; mean air concentration 0.3 - 2.3 ppm), and that dermal absorption of the liquid formulation was the major contributor.

Veulemans *et al* (1987) examined 2,654 air samples from 336 different industrial plants in Belgium for the presence of various glycol ethers. Though most exposure levels were below the current hygiene standards, in approximately 25% cases these levels were exceeded and sometimes extreme values were obtained (up to 370 ppm = 1,775 g/m^3). Values at painting and printing facilities ranged from 3.4-93.6 mg/m^3 (0.7 - 19 ppm) and 1.5 - 17.7 mg/m^3 (0.3 - 3.6 ppm) respectively. The median value from car repair areas was 5.9 mg/m^3 (1.2 ppm).

12.2.3. Exposure levels in the environment

Little data are available on the likely exposure in the environment. 2-Butoxyethanol has been reported in the ground water at contaminated sites, but not at other locations.

12.2.4. Measuring methods

The major methods for the analysis and detection of 2-butoxyethanol rely upon adsorption of the solvent onto a suitable adsorbent, desorption and analysis by gas chromatography.

The limit of detection of the available methods varies upon the volume of air drawn over the adsorbent, and the nature of the adsorbent but is typically 0.1 ppm to 2 ppm .

12.3. HEALTH SIGNIFICANCE

2-Butoxyethanol is absorbed via all routes, although dermal and inhalation exposure are likely to account for the principle human contact with this material. Inhalation is likely to be the major contributor to internal dose from vapour exposure to 2-butoxyethanol, and the dermal route from contact with the liquid.

The pharmacokinetics of 2-butoxyethanol have been studied in detail in male F344 rats, and similar metabolism has been observed in other species, including man. The main metabolic pathway for lower systemic doses following inhalation, dermal or oral exposure involves conversion to 2-butoxyacetic acid, which is rapidly excreted in the urine. There is an approximate first order relationship between 2-butoxyethanol exposure and 2-butoxyacetic acid excretion. Minor urinary metabolites in the rat include ethylene glycol, 2-butoxyethanol glucuronide, 2-butoxyethanol sulphate and small amounts of unchanged 2-butoxyethanol. In man, a glutamine conjugate of 2 butoxyacetic acid also has been reported.

Irrespective of the route of exposure, some of the absorbed 2-butoxyethanol is completely metabolised to CO_2, which is excreted in the expired air. At higher systemic doses, metabolism to butoxyacetic acid becomes saturated and glucuronide conjugation is more prominent, but even so butoxyacetic acid remains the major metabolite.

F344 rats show some age differences in metabolism, the younger animals excreting and/or further metabolising more 2-butoxyacetic acid, but no comparable data are available for man.

The acute toxicity of 2-butoxyethanol has been extensively studied in laboratory animals. The main cause of death from large doses of 2-butoxyethanol is narcosis. Intravascular haemolysis, caused by 2-butoxyacetic acid, is the principle toxic effect reported at lower doses in many species, as manifest by haemoglobinuria and secondary effects on kidney and other tissues. Older rats are more susceptible to these acute haemolytic effects. This is believed to be partly due to metabolic differences, and partly due to greater susceptibility of red cells from older rats to the haemolytic effects of 2-butoxyacetic acid.

The subchronic toxicity has been extensively investigated in several species using different routes of administration. Mice and rats appear to be highly sensitive to 2-butoxyethanol induced haemolysis; this determines the lowest observable effect levels. In these species, haemolysis is a transient effect predominantly seen in the early stages of exposure, and the replacing younger RBCs reticulocytes appear to be more resistant to 2-butoxyethanol. The generally recognised inhalation no effect level for haemolytic effects in rats is 25 ppm.

Guinea pigs and dogs, in contrast, show little or no effect on erythrocytes. For guinea pigs, no effect levels of 107 and 123 ppm have been reported. The observed effects at the next highest exposure level tested (203 and 250 ppm) were lung congestion, and an increase in

relative kidney weight in surviving animals, which may have been due to a decreased body weight gain. In human acute exposure studies, there were no reported effects at exposure levels up to 50 ppm. Higher exposures produced signs of respiratory irritation, discomfort and nausea, but not haemolytic effects. Nonetheless, haematological changes, coma, and metabolic acidosis have been reported in several attempted human suicide cases involving ingestion of large amounts of household products containing 2-butoxyethanol.

2-Butoxyethanol has been well studied for potential fertility and reproductive effects with a range of studies using various species, routes and protocols. The weight of evidence leads us to the conclusion that it has no effect on male reproductive performance. Whilst 2-butoxyethanol has shown some impact on female fertility, this was only at dose levels that caused maternal toxicity. It is not teratogenic, although embryo- and foeto-toxic effects have been observed at doses that were maternally toxic. The overall conclusion from these studies is that 2-butoxyethanol is not selectively toxic to the foetus or reproductive system.

From the available data it is concluded that butoxyethanol is not genotoxic. No chronic data are available, although rat and mouse inhalation studies are currently in progress.

12.4. FINAL EVALUATION AND RECOMMENDATION

Because of the uncertainties of extrapolating from animals to man, adequate human data should take precedence over animal data for developing human exposure standards. There is a considerable data base of human acute studies with 2-butoxyethanol.

12.4.1. Human Inhalation Exposure Studies with 2-Butoxyethanol

Haemolysis appears to be the primary, most sensitive indicator of 2-butoxyethanol toxicity in animals. This is an acute effect, often reversing with continued low exposure. Although no subchronic exposure studies have been performed in man, there are human acute exposure studies which were considered in this assessment.

There was no reported evidence of haemolysis, osmotic fragility (a precursor to haemolysis), or other systemic toxic effects when male and female human volunteers were exposed to either 100 or 195 ppm of 2-butoxyethanol for 8 h (Carpenter *et al*, 1956). It is relevant that haemoglobinuria was reported in rats exposed under the same conditions. The volunteers complained of discomfort from irritancy to the nose and eyes at both concentrations, and some individuals reported nausea and headache.

No irritancy, evidence of haemolysis (haemoglobinuria) nor other adverse health effects were reported in male volunteers, while exercising lightly and exposed to 20 ppm 2-butoxyethanol for 2 h (Johanson *et al*, 1986a).

No irritation, haemolysis or other systemic effects were reported in male volunteers exposed to 50 ppm 2-butoxyethanol vapour for 2 h (Johanson and Boman, 1991). The study compared uptake following concurrent inhalation and dermal (whole body) exposure. Each

exposure period lasted for 2 h, and was separated by a one hour rest. The results demonstrate that adverse effects are unlikely in human beings, even under exaggerated physical conditions.

Thus, human experience indicates that acute exposures to concentrations of 20 - 50 ppm are not overtly irritating. Concentrations of 100 ppm and above are clearly irritating and cause subjective toxic symptoms.

Because of the relatively short half-life of 2-butoxyacetic acid in man, systemic retention from one workday to the next is considered unlikely.

12.4.2. Determination of an animal NOEL

There are several studies in animals that have used inhalation, the route of concern for setting an indicative exposure value. It should be noted that all of these experiments involved whole body exposure to 2-butoxyethanol vapour. It therefore follows that the no effect levels so obtained reflect concurrent dermal and inhalation exposure to 2-butoxyethanol.

A no effect level of 25 ppm (Dodd *et al*, 1983) reported when rats were exposed for 90 d, 6 h/d by inhalation, appears to be the most reliable. The LOEL was 77 ppm, and the effects reported at this concentration were indicative of intravascular haemolysis (decreased RBC counts and blood Hb). At higher concentrations (up to 245 ppm in a 9 day study) the reported effects included haemoglobinuria, increased reticulocyte and lymphocyte counts, decreased bw gain and increased liver weight. A 14 day recovery period showed substantial reversal of the blood parameters.

In contrast, subchronic studies with species relatively insensitive to haemolysis have reported no effect levels (other than for respiratory irritation) as high as 107 ppm (Carpenter *et al*, 1956) and 125 ppm (Tyler, 1984) for guinea pig, and 100 ppm for dogs (Carpenter *et al*, 1956).

No longer-term inhalation studies are currently available for 2-butoxyethanol. However, red cell haemolysis in susceptible species such as rats and mice is a transient phenomenon seen only on the first few days of exposure, not thereafter (Dodd *et al*, 1983; Grant *et al*, 1985; Tyl *et al*, 1984). This reflects repopulation of the blood with erythrocytes resistant to 2-butoxyacetic acid-induced haemolysis. Thus, it is considered unlikely that a lower NOEL for haemolysis would be obtained following long term exposure of rodents or other, non-sensitive species.

12.4.3. Relevance of Haemolysis to Humans

Rats and mice are much more susceptible to 2-butoxyethanol-induced intravascular haemolysis than man. Humans beings exposed to concentrations as high as 200 ppm were not reported to exhibit haemoglobinuria, the initial most sensitive indicator of intravascular haemolysis (Carpenter *et al*, 1956). However, two human beings intentionally ingesting

formulated product containing 2-butoxyethanol did exhibit haemoglobinuria and decreased RBC counts, as well as metabolic acidosis and coma (Rambourg-Schepens *et al*, 1988; Gijsenbergh *et al*, 1989). These results indicate that at sufficiently high oral doses, haemolysis can occur in man.

12.4.3.1. Dose-response Relationship

The haemolytic effect of 2-butoxyethanol is mediated *in vivo* by its acid metabolite 2-butoxyacetic acid which causes swelling (a prehaemolytic effect) when rat RBC are incubated with 0.2 mM 2-butoxyacetic acid, and extensive haemolysis at 2 mM (Udden and Patton, 1994). Human RBCs, in comparison, are more resistant to these effects. Udden (1994) examined blood from several populations with a possible hypersusceptibility to 2-butoxyethanol-induced haemolysis. The blood samples came from elderly people, and sickle cell and hereditary spherocytosis patients. None exhibited haemolysis or prehaemolytic changes under conditions (2 mM 2-butoxyacetic acid) which readily haemolysed rat RBCs. There was increased cell volume with slight haemolysis in some individuals only at 8 mM 2-butoxyacetic acid (Ghanayem, 1989a). Thus, *in vitro* studies indicate that human red cells are at least 10 times, and may be up to 40 times less sensitive to 2-butoxyacetic acid-induced haemolysis than rat RBCs. Also, there appear to be no identified hypersusceptible populations of humans to 2-butoxyethanol-induced haemolysis.

12.4.4. Assessing a safe human dose

Assessing a safe human dose involves taking the no observed or lowest observed effect level from an animal study. Uncertainty factors are then applied. These are interspecies extrapolation (animal to man), intraspecies sensitivity (hypersusceptible individuals/populations), and a modification for time and route of application. In more sophisticated assessment by the inhalation route, physiological considerations are often used to calculate the equivalent human systemic dose at an animal no effect concentration. These principles are discussed below.

12.4.4.1. Interspecies Extrapolation

The default uncertainty factor of 10 for animal-to-man extrapolation, based on sensitivity to the toxic end point, need not be applied because studies show that human beings are not more sensitive than rats, the species for which the no effect level was determined. Theoretically, an uncertainty factor of 0.1 could be applied in recognition of the fact that human red cells are at least 10 time less sensitive to haemolysis than those from rats.

12.4.4.2. Intraspecies Sensitivity

The usual uncertainty factor of 10 for human variation (susceptible populations) need not be applied. Udden (1994) showed that RBCs from populations with certain haemolytic diseases were no more susceptible to 2-butoxyacetic acid-induced haemolysis than blood cells from healthy young humans.

12.4.4.3. Modification for time and route of exposure

Some regulatory authorities apply an additional uncertainty factor for subchronic to chronic exposure if the human exposure potential is for a significant portion of the life-span, as for example in long-term occupational exposure. The question to be addressed in the case of 2-butoxyethanol is whether such an uncertainty factor is needed. A no effect level of 25 ppm for haemolytic effects was determined in a subchronic (90 day) rat inhalation study. The haemolytic effect of 2-butoxyethanol exposure in susceptible animal species is usually manifest after the first few exposures, when older more susceptible red cells are haemolysed. The resulting younger red cells and reticulocytes are much less susceptible, and on continued exposure haemolytic effects and any sequelae may recover. On this basis, application of a subchronic to chronic uncertainty factor is inappropriate.

Human studies indicate that dermal uptake may account for about 75% of the total uptake during whole body exposure to 2-butoxyethanol vapour. In contrast, a PBPK model predicts that the dermal route may contribute no more than 20% to the overall systemic uptake. However, even if the actual dermal contribution accounts for 75% the actual measured blood concentrations of 2-butoxyacetic acid seen after exposure of human beings to 20 ppm 2-butoxyethanol vapour for 2 h were in the range of 22-60 μM (see 7.1.4). This is in agreement with a PBPK model predicting human 2-butoxyacetic acid blood concentrations of approximately 30 μM at similar exposure levels. This finding indicates irrespective whether dermal uptake is the major contributor to the overall systemic uptake in man during whole body exposure to 2-butoxyethanol vapour that the effective blood concentration of the haemolytic metabolite 2-butoxyacetic acid is several 100 times below those 2-butoxyacetic acid concentrations (8,000 μM) which caused minimal prehaemolytic effects in human beings RBC *in vitro* (see 12.4.4.4). Thus the application of an uncertainty factor for the route of exposure is not appropriate. Furthermore it should be noted that all animal experiments involved whole body exposure to 2-butoxyethanol vapour. It follows therefore that the no effect level obtained (25 ppm) reflects concurrent dermal and inhalation exposure to 2-butoxyethanol vapour. This further suggests that an additional uncertainty factor for the occurrence of skin absorption is not required.

12.4.4.4. Physiological considerations

In arriving at an acceptable human exposure standard, information derived from a PBPK model was used to compare dosimetry of 2-butoxyethanol in animals and man. Since the "Area Under the blood concentration time Curve" (AUC) is the integral of the toxicokinetic behaviour of a chemical, this parameter rather than peak-blood levels has been used as internal dose surrogate for interspecies comparison.

The PBPK model predicts that human beings exposed under steady state conditions to 22 ppm 2-butoxyethanol will show an AUC-value of 2-butoxyacetic acid in blood similar to that seen in rats exposed to the NOEL of 25 ppm.

Furthermore the peak concentration of the haemolytic metabolite in human blood at this exposure level (33 μM) is several hundred fold below that likely to cause pre-haemolytic effects in human RBC *in vitro* (8,000 μM).

These physiological considerations confirmed the view that an interspecies assessment factor is not required (see 12.4.4.1).

12.4.5. Conclusion

The available human data, extrapolation from animal studies and information from PBPK-modelling supports an exposure standard in the range currently employed by most countries i.e. 20 ppm (8 h TWA). The data do not indicate that a lower inhalation exposure level would be more protective of human health.

Respiratory irritation may occur above 50 ppm; a short-term exposure limit of 50 ppm (15 min) is recommended.

A skin notation is recommended because of the potential for significant dermal absorption of liquid 2-butoxyethanol.

12.4.6. Recommendations

Indicative Limit Value 20 ppm (8 h TWA)
Short-term Limit Value 50 ppm (15 min TWA)
Skin Notation.

13. SUPPLEMENT-2-Butoxyethylacetate

13.1. SUBSTANCE IDENTIFICATION

13.1.1. Identity

2-Butoxyethylacetate has the chemical structure $CH_3CH_2CH_2CH_2OCH_2CH_2$-OCO-$CH_3$.

The IUPAC name is 2-n-Butoxyethylacetate and the Chemical Abstracts Service 8th and 9th nomenclature is Ethanol, 2-butoxyacetate. For the purposes of this Criteria Document the name 2-Butoxyethylacetate is used.

Trade names and synonyms are given in table VII.

Table VII
2-Butoxyethylacetate - Synonyms and Trade Names

Name	Numbers	Synonyms	Trade Names
2-Butoxyethanol acetate	EINECS[1)] 203-933-3	Ethanol, 2-butoxyacetate	Butyl Cellosolve Acetate
		2-Butoxyethylacetate 2-n-Butoxyethyl acetate 2-Butoxyethanol acetate	'Embkanol' AEG
	CAS 112-07-2	Butyl Glycol Acetate	Butyl 'Ethoxol' acetate
	RTECs KJ8925000	Ethylene Glycol Butyl Ether Acetate (EGBEA)	

[1)] European Inventory of Existing Chemical Substances

2-Butoxyethylacetate is classified and labelled in the EC as below:

EC Classification Xn (harmful)

EC Labelling
Risk Phrases: R20/21
Harmful by inhalation and in contact with skin

Safety Phrases: S24
Avoid contact with skin

13.1.2. Chemical and Physical Properties

2-Butoxyethylacetate is a colourless liquid slightly soluble in water and soluble in most organic solvents. Other chemical and physical properties are listed in table VIII.

Table VIII
Chemical and Physical Properties of 2-butoxyethylacetate

Properties	Value[1)]
Molecular weight	160,2
Molecular formula	$C_8H_{16}O_2$
Melting point °C	-64
Boiling point °C	192 (at 1014 hPa)
Vapour pressure hPa at 20°C	0,4
Water solubility at 20°C	1,5% w/w
Conversion factors	1 ppm = 6,65 mg/m^3 (20°C, 1014 hPa) 1 mg/m^3 = 0,15 ppm (20°C, 1014 hPa)

[1)] Rowe and Wolf (1982)

13.2. TOXICOKINETICS

The half life of 2-butoxyethylacetate in rat plasma *in vitro* is approximately 1 minute (Hoffmann and Jäckh, 1985).

The molecule is rapidly cleaved, presumably by esterases, into 2-butoxyethanol and acetate. For that reason the systemic toxicity of butoxyethylacetate is practically equivalent to that of 2-butoxyethanol. The effective doses and no adverse effect levels may be regarded as nearly identical on a molar basis.

According to acute and subacute toxicity studies (Smyth *et al*, 1962, Truhaut *et al*, 1979) the material is adsorbed through the skin in significant amounts.

13.3. TOXICODYNAMICS

13.3.1. Acute toxicity

Smyth *et al* (1962) reported an oral LD_{50} value of 7.46 ml/kg bw in male rats. The authors did not report age or weight of the animals used in this investigation.

Truhaut *et al* (1979) obtained the following LD_{50} values in rats (220 - 240 g):

3,000 ± 300 mg/kg bw (males)
2,400 ± 299 mg/kg bw (females)

In rabbits (2.2-2.5 kg), a dermal LD_{50} value of ~1,500 mg/kg bw was found (Truhaut *et al*, 1979).

Symptoms in rats and rabbits were haemolysis, haemoglobinuria and metabolic acidosis. Histologic examination showed renal tubular nephrosis throughout all dose groups with increasing severeness and progressive recovery in surviving animals (Truhaut *et al*, 1979).

A single 4-h inhalation exposure on a vapour-saturated atmosphere of 2-butoxyethylacetate (~ 400 ppm) was without effect on rats and rabbits (Truhaut *et al*, 1979). After 8 h inhalation at the same concentration some haemoglobinuria was observed (Smith *et al*, 1962).

13.3.2. Irritation

The neat liquid was practically non irritating to rabbit skin and eye (Truhaut *et al*, 1979) or human skin (Jacobs *et al*, 1989).

13.3.3. Sensitization

No data available.

13.3.4. Subacute/subchronic toxicity

Haemoglobinuria was reported in rats repeatedly exposed to saturated atmosphere (approx. 400 ppm) for 4 h/d, 5 d/wk over 2 weeks. After 3 weeks, decreased RBC count and blood haemoglobin concentration was observed. These effects were not seen at 100 ppm. Thymus, lymph nodes and bone marrow were not examined in this study (Truhaut *et al*, 1979).

Haemoglobinuria was also seen in four rabbits exposed to a vapour saturated atmosphere for 2 weeks. Two animals died in week 4, the other animals recovered within a 1 week post-observation period (Truhaut *et al*, 1979).

No treatment-related effects were seen in rats and rabbits following repeated exposure to 100 ppm 2-butoxyethylacetate for 10 months. Histological examination showed discrete renal lesions (Truhaut *et al*, 1979).

The results are summarised in table IX.

13.3.5. Carcinogenicity/Genotoxicity/Reproduction/Developmental Toxicity

No data were identified.

Table IX
Effects of 2-butoxyethanol acetate on the haematopoietic system of experimental animals

Rat - oral	Dose	Effect
Wistar, male and female	Lethal doses (~3 g/kg bw for males, 2.4 g/kg for females)	Haemoglobinuria and/or haematuria present up to 1 wk post-treatment. Kidneys hypertrophic and "dilated with blood" (Truhaut *et al*, 1979).
Rat - Inhalation		
Wistar, male and female	Saturated vapour, single 4 h exposure	Haematuria not present (Truhaut *et al*, 1979).
Wistar, male and female	Saturated vapour, 4 h/d, 5 d/wk for 1 month	Slight haemoglobinuria and/or haematuria from wk 2 onwards. No gross pathological lesions noted (Truhaut *et al*, 1979).
Wistar, male and female	100 ppm, 4 h/d, 5 d/wk for 10 months	No effects on RBC count, Hb concentration or leukocyte count. No haematuria. No gross lesions (Truhaut *et al*, 1979).

Rabbit - dermal	Dose	Effect
New Zealand, male and female	Lethal dose approx. 1.5 g/kg bw occl. after 24 h	Haemoglobinuria and/or haematuria, with marked reduction in RBC count and Hb concentration, seen in some animals from all groups 48-72 h post-exposure. Values normal within 8-14 d (Truhaut *et al*, 1979).
Rabbit - Inhalation		
New Zealand, male and female	Saturated vapour, single 4 h exposure	Slight haematuria during 24 h following exposure (Truhaut *et al*, 1979).
New Zealand, male and female	Saturated vapour, 4 h/d, 5 d/wk for 1 month	Moderate haematuria and/or haemoglobinuria from wk 2. RBC count and Hb concentration decreased in 2 out of 4 rabbits during wk 4. Kidneys hypertrophic and "swollen with blood" (Truhaut *et al*, 1979).
New Zealand, male and female	100 ppm, 4 h/d, 5 d/wk for 10 months	No haematuria or effects on RBC, Hb or leukocyte counts. No macroscopic lesions at necropsy (Truhaut *et al*, 1979).

13.4. SUMMARY AND CONCLUSION

The vapour pressure and volatility of 2-butoxyethylacetate are lower than that of 2-butoxyethanol. 2-Butoxyethylacetate appears to show less potential to cause dermal and eye irritation than 2-butoxyethanol.

2-Butoxyethylacetate is rapidly hydrolysed in blood to 2-butoxyethanol and acetic acid. The latter enters the intermediary metabolism via the Krebs cycle, as does endogenous acetate, and is metabolized to carbon dioxide, which is exhaled.

The health effects of the resultant 2-butoxyethanol originating from the hydrolysis of 2-butoxyethylacetate are considered equivalent to those occurring after exposure to 2-butoxyethanol itself. Therefore the safety assessment described for 2-butoxyethanol (see chapter 12.4) can also be applied to 2-butoxyethylacetate.

13.5. RECOMMENDATIONS

Indicative Limit Value	20 ppm (8 h TWA)
Short-term Limit Value	50 ppm (15 min TWA)
Skin notation	

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14.3. DATABASES CONSULTED

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BASF Internal data base. Product profiles BASF AG 67056 Ludwigshafen

14.4. REFERENCES NOT QUOTED IN THE DOCUMENT

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